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Key prokaryotic populations contributing to carbon flow in an agricultural soil food web

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Prosit!

(lat.: Es möge nützen!)

Abstract

Microorganisms determine the turnover, fluxes and mineralisation of organic carbon in soils and they are crucial for soil food webs. A detailed understanding of the trophic interactions in soil food webs is needed in order to gain more comprehensive insights into terrestrial carbon cycling. The DFG Research Unit FOR 918 "Carbon flow in belowground food webs assessed by isotope tracers" was initiated to provide such integrated knowledge on an exemplary soil food web, with all its involved organisms (from micro- to macrofauna), their trophic links and the carbon flow between them. Despite their importance, microorganisms and especially bacteria are not well represented in soil food web concepts up to now, as they are mostly considered as mere undefined biomass. Here, I aimed to open this bacterial 'black box', to identify specific key bacterial populations involved in a soil food web based on plant-derived carbon, and to investigate their abundance and distribution in an agricultural field.

I hypothesised 1) that specific bacterial populations are distinctly involved in the degradation of both labile and more recalcitrant plant-derived substrates; 2) that these specific key populations are not evenly distributed in the soil in space and time, but they are subject to characteristic heterogeneity due to soil compartments, soil depth, and seasons. Moreover, I investigate the mobilisation and transport of natural soil bacteria by seepage water and postulate that 3) distinct top soil bacteria are selectively mobilised, and that transported bacteria contribute noticeably to carbon flux into deeper soil layers.

The overall bacterial community structure of the exploratory agricultural field was investigated by 16S rRNA gene and 16S rRNA T-RFLP fingerprinting, amplicon pyrotag sequencing and qPCR. Variation of community composition was analysed for soil compartment (bulk soil, rhizosphere and root surface), depth, seasonal sampling time points and cultivation treatment (with or without litter amendments). Taxa of key bacterial populations involved in the food web were identified by RNA stable isotope probing (SIP) in microcosm experiments with ^{13}C-labelled substrates of varying complexity and recalcitrance: rhizodeposits, root and leaf litter, glucose and cellulose as model analogues, as well as bacterial biomass. Subsequently, the abundance and distribution of experimentally identified bacterial taxa was assessed *in situ* by a unique combination of pyrotag sequencing and qPCR. Bacterial transport into deeper soil by

seepage water was investigated via fresh lysimeter samples, and respective soil samples obtained from the same depths.

The bacterial community in the field was mostly affected by soil compartment and depth. Season and cultivation treatment had minor, but significant influence on community structure. All ^{13}C-substrates were utilised by specific, only partially overlapping bacterial populations. Substrate quality and complexity did indeed define the identity and diversity of primary consumers. While glucose carbon was mainly assimilated by *Arthrobacter* spp. and *Micrococcaceae*, cellulose was mainly degraded by *Cellvibrio* spp., *Flavobacterium* spp. (initially) and *Streptomycetaceae* (later). Bacteria involved in leaf and root litter degradation were affiliated to *Cellvibrio*, *Flavobacterium, Mucilaginibacter* and *Cytophaga* spp. (early time point), as well as *Ohtaekwangia* spp. and the *Polyangiaceae* (secondary labelling). Rhizodeposit consumers were diverse but much less strongly labelled compared to detritussphere bacteria, comprising mainly species of the genera *Opitutus, Mucilaginibacter, Massilia* and *Ohtaekwangia,* some of them not identified as rhizodeposit utilisers to date. In contrast, it was not possible to elucidate secondary inter-microbial trophic interactions with bacterial biomass amendment, due to unexpected high survival rates of the initially added labelled soil bacteria. In the field, identified rhizodeposit consumers were indeed highly abundant in the rhizosphere and at the root surface in summer, whilst litter degraders were most abundant on decaying roots in winter. In contrast, identified glucose utilisers (*Actinobacteria*) were quite evenly distributed, and also abundant in bulk soil. Comparing bacteria in lysimeter water and soil samples after snowmelt, a selective mobilisation of distinct root-associated bacterial populations was revealed, providing an interesting new perspective of potential mechanisms linking top and subsoil microbial communities.

This work clearly shows that bacteria are not adequately incorporated in current food web models. Their role in the turnover of both labile and recalcitrant organic substrates needs to be reconsidered. Specific bacterial populations, heterogeneous in space and dynamic in time, have to be considered as driver of carbon fluxes from distinct plant-derived carbon sources in soil. Thus, the mostly static organismic interaction networks in current food web concepts need to evolve for an adequate integration of these findings. These insights are crucial to improve our current perspective of bacterial functional diversity and organismic carbon fluxes in soil food webs.

Zusammenfassung

Mikroorganismen bestimmen maßgeblich den Umsatz, die Stoffflüsse und die Mineralisierung von organischem Kohlenstoff in Böden und sind von zentraler Bedeutung für Bodennahrungsnetze. Für ein umfassenderes Verständnis des terrestrischen Kohlenstoffkreislaufs, müssen auch die trophischen Interaktionen des Bodennahrungsnetzes bekannt sein. Deshalb wurde die DFG Forschergruppe FOR 918 („Kohlenstoffflüsse in unterirdischen Nahrungsnetzen ermittelt durch Isotopensignaturen“) gegründet, um detaillierte Erkenntnisse über alle betroffenen Organismen (von Mikro- bis Makrofauna) in einem exemplarischen Bodennahrungsnetz zu gewinnen sowie deren trophischen Verbindungen und die entsprechenden Kohlenstoffflüsse aufzuzeigen. Ungeachtet ihrer Bedeutung werden Mikroorganismen und vor allem Bakterien in derzeitigen Bodennahrungsnetzmodellen nicht detailliert berücksichtigt und meist nur als bloße Biomasse dargestellt. Mit dieser Arbeit möchte ich diese bakterielle ‚black box‘ öffnen, die in einem auf Pflanzenkohlenstoff basierenden Bodennahrungsnetz beteiligten spezifischen bakteriellen Schlüsselpopulationen identifizieren und deren Verteilung und Häufigkeit in einem Acker-Boden untersuchen.

Dazu stelle ich folgende Hypothesen auf: 1) Am Abbau von sowohl labilem als auch eher rekalzitranten Substraten sind spezifische bakterielle Populationen maßgeblich beteiligt. 2) Diese spezifischen Schlüsselpopulationen sind nicht räumlich und zeitlich gleichmäßig im Boden verteilt, sondern unterliegen charakteristischen Schwankungen hinsichtlich Bodenkompartiment, Bodentiefe und Jahreszeit. Außerdem habe ich die Mobilisation und den Transport von natürlichen Bodenbakterien durch Sickerwasser untersucht. Diesbezüglich postuliere ich: 3) Bestimmte Oberbodenbakterien werden selektiv mobilisiert und transportierte Bakterien tragen merklich zum Kohlenstoffffluss in tiefere Bodenschichten bei.

Die Struktur der gesamten bakteriellen Gemeinschaft wurde mit 16S rRNA Gen und 16S rRNA T-RFLP Fingerprinting, Amplikon-Pyrosequenzierung und qPCR hinsichtlich Veränderungen in der Zusammensetzung verursacht durch Bodenkompartiment (Rhizosphäre, Wurzeloberfläche, nicht durchwurzelter Boden), Bodentiefe, Jahreszeit und Anbaumethode (mit und ohne Streuauftrag) untersucht. Die Taxa der am Nahrungsnetz beteiligten bakteriellen Schlüsselpopulationen wurden mit ^{13}C markierten Substraten von verschiedener Komplexität und Rekalzitranz (wurzelbürtige Substanzen, Pflanzenmaterial der Wurzel und Blätter, Glukose und Zellulose) in Mikrokosmenexperimenten mit RNA-

stable isotope probing (SIP) identifiziert. Anschließend wurden die Verteilungen und Häufigkeiten dieser experimentell identifizierten Bakterien im Feld mit einem neuartigen Ansatz, der Amplikon-Pyrosequenzierung und qPCR verbindet, ermittelt. Der Bakterientransport in tiefere Bodenschichten durch Sickerwasser wurde mittels frischer Lysimeterproben und Proben aus entsprechenden Bodentiefen untersucht.

Die bakterielle Gemeinschaft vom Feld wurde hauptsächlich durch Bodenkompartiment und Bodentiefe beeinflusst. Jahreszeit und Anbaumethode hatten je einen geringen, aber signifikanten Einfluss. Die jeweiligen ^{13}C-Substrate wurden von spezifischen, nur teilweise sich überschneidende bakteriellen Populationen verwendet. Sowohl Substratkomplexität als auch -rekalzitranz waren bestimmenden Faktoren für Identität und Diversität der Primärkonsumenten. Glukose wurde vor allem von *Arthrobacter* spp. und *Micrococcaceae* verwendet, Zellulose hingegen anfänglich von *Cellvibrio* spp., *Flavobacterium* spp., später dann von *Streptomycetaceae*. Am Abbau von Pflanzenmaterial (Wurzel und Blatt) waren anfänglich *Cellvibrio* spp., *Flavobacterium* spp., *Mucilaginibacter* spp. und *Cytophaga* spp. beteiligt, später waren es *Ohtaekwangia* spp. und *Polyangiaceae* spp.. Die bakteriellen Nutzer wurzelbürtiger Substrate waren sehr divers, aber weniger stark markiert als die Nutzer von Detritussubstraten. Hier wurden vor allem Arten der Gattungen *Opitutus*, *Mucilaginibacter*, *Massilia* und *Ohtaekwangia* identifiziert; davon sind einige noch nicht als Rhizobakterien bekannt. Bei dem Experiment mit markierter Biomasse konnten kaum trophische Interaktionen zwischen Mikroorganismen festgestellt werden, da die Überlebensrate der zugegebenen markierten Bodenbakterien sehr hoch war. Die identifizierten Nutzer wurzelbürtiger Substrate waren am häufigsten in der Rhizosphäre und an der Wurzeloberfläche im Sommer, die Pflanzenmaterialverwerter dagegen kamen vor allem an verrottenden Wurzeln im Winter vor. Identifizierte Glucosenutzer (*Actinobacteria*) hingegen waren ziemlich gleichmäßig verteilt und waren auch in von Wurzeln unbeeinflussten Bodenbereichen häufig anzutreffen. Beim Vergleich der Bakterien der Lysimeter- und Bodenproben nach der Schneeschmelze wurde ersichtlich, dass bestimmte wurzelnahe Bakterien selektiv mobilisiert wurden. Dies eröffnet neue Perspektiven auf möglichen Mechanismen, durch die die Gemeinschaften der Ober- und Unterbodenbakterien miteinander verbunden sind.

Diese Arbeit stellt deutlich heraus, dass Bakterien in derzeitigen Nahrungsnetzmodellen nicht ausreichend berücksichtigt werden. Zum einen muss ihre Rolle beim Abbau sowohl labiler als auch rekalzitranter Substrate überdacht werden. Zum anderen müssen spezifische bakterielle Populationen mit eigenständigen zeitlichen und räumlichen

Dynamiken als eine Antriebskraft für Kohlenstoffflüsse im Boden, ausgehend von verschiedenen pflanzlichen Kohlenstoffquellen, berücksichtigt werden. Die bisher eher statische Betrachtung der Wechselwirkungen von beteiligten Organismen in derzeitigen Nahrungsnetzmodellen muss weiter entwickelt werden um diese Ergebnisse angemessen einzubinden. Diese Erkenntnisse tragen entscheidend dazu bei, den derzeitigen Blickwinkels auf die funktionelle Vielfalt von Bakterien und Kohlenstoffflüssen zwischen Organismen in Bodennahrungsnetzen zu verbessern.

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1. Introduction

1.1. The role of soils in carbon cycling

Global warming and increased carbon dioxide emissions make the understanding of carbon cycling and carbon sinks very urgent scientific objectives. Globally, carbon is emitted as carbon dioxide into the atmosphere mediated by fuel combustion, organismic respiration, erosion and deforestation and is fixed again mainly by photosynthesis from plants, algae and phototrophic bacteria (Fig. 1). Humans interfere progressively with the natural carbon balance and shift the equilibrium towards atmospheric carbon. The atmospheric carbon dioxide stock is increased by 3.5 Pg (billion tonnes) of carbon each year due to combustion of fossil fuels and intensive land use (Srivastava *et al.* 2012).

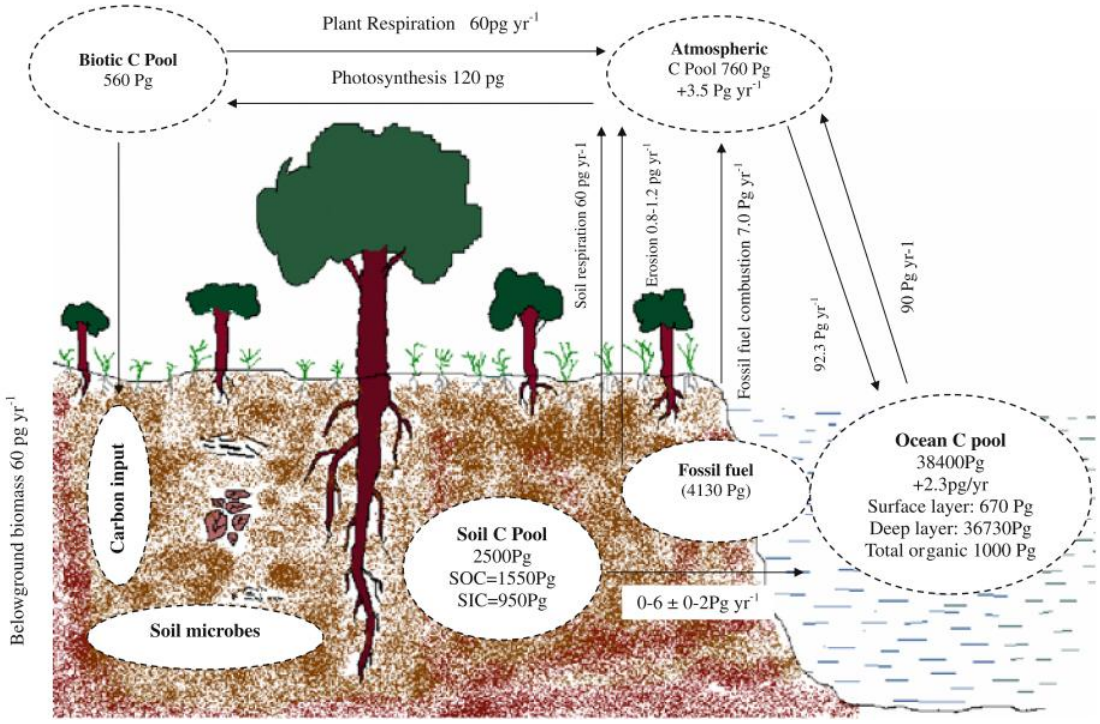

Fig. 1: Global sources and sinks of carbon in the pedosphere, atmosphere and hydrosphere. (taken from Srivastava *et al.* 2012).

1.1.1. Carbon pools in soils

Soils are the third biggest carbon pool on earth (Lal 2004) and sequester 20 to 30 % of anthropogenic carbon emissions (Singh *et al.* 2004). They can both function as source and sink for carbon dioxide depending on whether soil respiration or carbon input predominate. Particularly, changes in climate and land use can alter the exchange equilibrium between soil and atmosphere (Paterson *et al.* 2011). Thus, detailed knowledge about the soil carbon

cycle and the mechanisms of carbon mineralization, mobilization and distribution in soils are very important to understand global carbon cycling. Also, better prediction and management of the impacts of global change on the soil carbon pool can be achieved if the processes of carbon flux in soils are known.

On a global scale, soil carbon (2500 Pg) can be divided into soil inorganic carbon (SIC, 950 Pg) and soil organic carbon (SOC, 1550 Pg) (Dungait *et al.* 2012). SOC is mostly comprised in soil organic matter (SOM) which is best specified as a complex mixture of plant, faunal and microbial residues at various stages of decay (Kelleher *et al.* 2006, Miltner *et al.* 2012).

SOM is not evenly distributed in soils but mostly decreases with soil depth. Soils are vertically structured into horizons according to physical properties caused by different factors of pedogenesis. A common horizon pattern observed in temperate soils displays the O-A-B-C sequence. Here, a relative thin O horizon, with very high SOM concentration (> 30 %) is located above the A horizon or top soil with intensely decomposed organic matter where most biological activity occurs. Further down, the B horizon or subsoil is composed of mineral layers from the even deeper C horizon (parent rock) blended with accumulations from the A horizon and low SOM concentration (Gisi *et al.* 1997).

1.1.2. Control of SOM turnover by microorganisms

Stability of soil organic matter is of great importance as it is the largest carbon pool in soils and crucial for the net carbon flux to and from the atmosphere (Dungait *et al.* 2012). SOM can be classified into three phases regarding its stability: the active or labile carbon pool (turnover rate of 1 - 10 years), the intermediate (10 - 100 years) and the passive pool (100 - >1000 years) (von Luetzow *et al.* 2006). SOM stability is controlled by selective preservation due to recalcitrance, spatial inaccessibility and interaction with mineral surfaces (Sollins *et al.* 1996). Recalcitrance comprises all degradation barriers due to molecular characteristics of the compounds. Spatial inaccessibility describes physical protection of SOM through occlusion in soil aggregates. Interaction of SOM with mineral surfaces can alter the surface of macromolecules towards higher hydrophobicity which makes them less accessible for microorganisms. (Bachmann *et al.* 2008, Marschner *et al.* 2008)

Although SOM carbon initially originates mostly from plants who release carbon compounds as rhizodeposits and litter to the soil (Kögel-Knabner 2002), composition and stability of SOM is largely controlled by the degrading organisms and particularly by the

involved microorganisms in this habitat (Nielsen *et al.* 2011). Organic carbon compounds can be respired and mineralised by diverse belowground organisms (bacteria, fungi, protists, soil fauna), but also sequestered in the form of recalcitrant soil organic matter. SOM is selectively degraded by organisms due to transport restrictions, C:N and C:P ratios and oxygen limitation (Ekschmitt *et al.* 2008). Microorganisms are of capital importance for SOM degradation and sequestration as they are responsible for 85 - 90 % of the decomposition of organic materials in soils (Ekschmitt *et al.* 2008). Due to degrading activity in soils 50 % - 75 % of freshly introduced rhizodeposits were reported to be respired directly, 4 % - 9 % were incorporated in microbial biomass and 20 % - 45 % were allocated in the soil as SOM (Marx *et al.* 2007). Also, about 30% of maize litter carbon was stored in the soil as SOM (Flessa *et al.* 2000).

Furthermore, SOM is not only catalytically transformed but largely made from decayed microorganisms. This microbial contribution to SOM genesis has been underestimated for long (Simpson *et al.* 2007) but up to 80 % of SOM can origin directly from microbial biomass (Liang *et al.* 2011a). Whereas lignin cannot be considered as recalcitrant *per se*, microbial macromolecules like chitin and murein are very stable in soils (von Luetzow *et al.* 2006). Also, cell envelope fragments, proteins and especially peptides tend to interact with soil particles and thereby become very stable in soils (Fan *et al.* 2004, Kögel-Knabner 2002, Miltner *et al.* 2012).

In summary, the amount of carbon stored in soils is mainly regulated through mineralisation and immobilization of SOM by bacteria and fungi and depends directly upon the abundance and activity of the microbial population in soil.

1.2. Soil food webs

As SOM and carbon turnover in soils is determined by the converting organisms, their feeding interactions are crucial to understand carbon fluxes in soils. The entire interaction net in a habitat is commonly referred to as food web.

Originally elaborated and used for aboveground habitats, food web studies illustrate the feeding interactions of the organisms in a given system and their mutual dependence. Therefore, food webs are the key to understand matter and energy fluxes in ecosystems.

With increasing interests in global nutrient cycling and decomposition processes, food web principles were also applied to soil organisms (first: Hunt *et al.* 1987). “Soil food webs sequester carbon, cycle nutrients, maintain soil health to suppress pathogens, help plants tolerate abiotic and biotic stress, and maintain ecosystem resilience and sustainability”

(Chakraborty *et al.* 2012). Due to those vital ecosystem services, it is an important task to understand soil food web functioning, identify key stone organisms, their interaction and the carbon flow within them, particularly as climate change is predicted to alter soil diversity which may have substantial effects on soil carbon cycling and flow (Nielsen *et al.* 2011).

1.2.1. Overview and definitions

Generally, organisms within a food web are grouped into trophic levels according to their function and not by phylogenetic classification (Scheu 2002). Typical functional groups are primary producers who synthesise biomass from inorganic compounds (mostly plants) and primary consumers who feed on the producers as herbivores. Secondary consumers subsist preliminary on primary consumers as predators and decomposers utilise dead materials from both flora and fauna. Top predators have no natural enemies and stand on the highest level of the food web. Still, their dead biomass is recycled into the food web by decomposers again (Hui 2012).

There are two main mechanisms by which trophic interactions directly control food web member populations: top-down and bottom-up control. Top-down means the regulation of the respective resource population by the consumer and bottom-up describes the effect of resource availability on the consumer's population. Soil food webs are mostly regulated by bottom-up and not top-down effects because they mainly depend on decomposition of plant materials (Mikola *et al.* 1998, Shurin *et al.* 2006). But there are some possibilities for top-down control as well, as quality and amount of litter and exudates can be influenced indirectly by mineralisation rates and supply of essential minerals like nitrate and phosphate (Moore *et al.* 2003).

Soil food webs comprise a huge diversity of organisms. To give an overview, only very general phylogenetic groups are considered here: besides the microorganisms – bacteria, fungi and protozoa (mostly flagellates, amoeba and ciliates) –, arthropods (e.g. collembolan, mites and isopoda) nematodes and annelids (mostly earthworm and other *Enchytraeidae*) are the main members of the soil food web (see also Fig. 2).

However, investigating soil food webs is not as facile as for aboveground habitats due to some inherent restrictions. Soil organisms and their trophic interaction are difficult to observe since soil is an opaque environment and most of the resident organisms are of microscopic size (Albers *et al.* 2006). In addition, soil food webs are often very complex as the spatio-temporal heterogeneity of the soil itself provides many opportunities for niche

formation and allows many trophic competitors to coexist, promoting the high biodiversity in soils (Ettema *et al.* 2002). Moreover, various multi-trophic interactions are frequent in soil food webs and members of a lower trophic level, as for example bacteria and fungi, can feed on their predators as well (Chakraborty *et al.* 2012). Therefore, very sophisticated approaches are required to identify food web members and their trophic interactions. Tracing of stable isotopes turned out to be very useful for this. The stable but natural rare carbon isotope ^{13}C can be used either directly via labelled substrate or indirectly by tracing the changes of isotope ratios along the food chain (Staddon 2004, Wada *et al.* 1991).

Stable isotopes were traced in several studies to investigate soil food webs. However, so far, mostly detrital food webs have been explored neglecting root exudates as primary carbon source (Paterson *et al.* 2011, Pollierer *et al.* 2012, Scheu 2002). Furthermore, almost all studies describe soil food webs as static interaction networks without considering temporal and spatial heterogeneity which occur in natural soil habitats (Berg *et al.* 2007). The impact of such dynamics like soil depth and seasonality on soil food webs still needs to be clarified.

1.2.2. Soil food web models: state of the art

Interactions and relationships of organisms in a food web are depicted in food web models. Topological food web models qualitatively represent the feeding interactions between its members, whereas interaction strength webs focus on the impact the feeding relationship has on the organism populations (Scheu 2002). With regard to nutrient cycling, quantitative energy flow webs are more appropriate, as they comprise carbon amounts for the trophic interactions (Bersier *et al.* 2002). Tracking of carbon fluxes is a suitable method to quantify energy flow in a food web because most non-photoactive organisms derive energy from converting organic compounds with higher energy level into compounds with lower energy level.

The role of fauna in soil food webs is already well understood and described in food web models. Soil microorganisms, however, and especially bacteria are still treated as "black box" – mere biomass where carbon flows through without further trophic levels and interactions (Fig. 2, Allison *et al*. 2008).

In the last four decades, food web models have been improved only little regarding the detailed implementation of microorganisms let alone their intra-group interactions (Manzoni *et al.* 2009). The model with the highest resolution divides microorganisms in no

more than three guilds defined by substrate utilisation (labile, moderate and recalcitrant) (Moorhead *et al.* 2006).

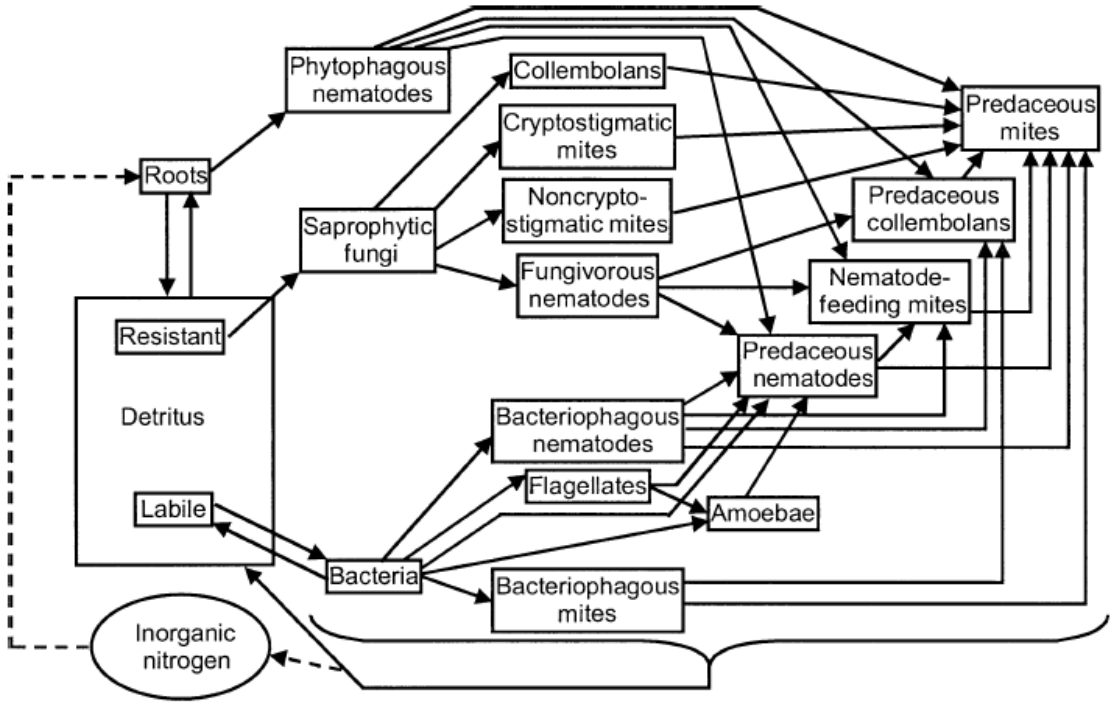

Fig. 2: Example of a belowground food web from the rhizosphere of the shortgrass prairie of Colorado (from Moore *et al.* 2003)

Furthermore, functional diversity of bacteria is neglected so far, as well. It is widely supposed that bacteria introduce only carbon from labile litter compounds into the detritus food web, whereas more recalcitrant substrates are utilised by fungi. Because of feeding preferences (bacteria or fungi) of organisms at the next trophic levels, distinct energy channels with almost no trophic interactions were proposed (Moore *et al.* 1988). Because of higher turnover rates of bacteria than of fungi, this led to the separation of a "fast" (bacterial) and "slow" (fungal) energy channel in soil food webs (Moore *et al.* 2003). According to that model, in the 'fast' ('slow') energy channel carbon from labile (recalcitrant) substrates is assimilated by bacteria (fungi) which are prey of bacteriovores (fugivores). This classification may not be operational for all soil food webs. The formation of such separated "energy channels" can be very weak (Witt *et al.* 2010) or do not work in parallel but interact, as omnivory is more common among soil fauna in natural environments than observed experimentally (Crotty *et al.* 2011, Ngosong *et al.* 2009, Pollierer *et al.* 2012).

Those incompatibilities of model and reality may be resolved if microorganisms and particularly, bacteria would not only be treated as one single group at one trophic level (Mikola *et al.* 1999). Instead, the high functional and phylogenetic diversity of soil bacterial communities should be regarded in soil food web models.

Often, implementation of microbial diversity in soil food web models is considered unnecessary as it is assumed that soil bacteria act as a constant and behave either resistant, resilient or at least show functional redundancy to disturbances due to their mere high diversity. Both persistence and resilience of soil bacterial communities towards ecological changes could be disproved by Allison et al. and even functional redundancy was challenged (Allison *et al.* 2008). Assuming that not 'everything is everywhere' (Martiny *et al.* 2006, O'Malley 2007) because of spatial restrictions and only limited dispersion (Ekschmitt *et al.* 2008, Hansel *et al.* 2008), it is possible that microbial populations responsible for certain soil functions are inhibited due to environmental disturbances. Particularly, functions not widely spread among microorganisms like nitrification, nitrogen fixation and degradation of humic acids can be affected, if relevant microbial populations decrease or change (McGuire *et al.* 2010). And indeed there is a lot of evidence that changes in microbial communities have impact on soil functioning, contradicting the redundancy hypothesis. For example, both soil organic matter and litter degradation rates change under different microbial communities (Bray *et al.* 2012, Garcia-Pausas *et al.* 2011) and soil functioning was affected by distinct compositions of the microbes because of different climatic conditions and land use variations (Bissett *et al.* 2011, Sherman *et al.* 2012).

Demands increased to consider and implement microbial communities into food web models: Bacteria could be divided into active and inactive biomass because only the former introduce carbon into the food web (Nannipieri *et al.* 2003). Moreover, key players of functional groups could be implemented to account for varying turnover rates of different degrading species (Allison *et al.* 2008). Anyhow, it should always be tested experimentally if microbial diversity is important to the processes modelled (McGuire *et al.* 2010). Therefore, research is needed to identify and quantify the specific microbial organisms that utilise plant and soil carbon sources (Paterson *et al.* 2009).

1.2.3. Bacteria in soil food webs

To account for different functions in the food web, bacteria are not only classified phylogenetically, but also functionally. Beside categorizing into groups according to

substrate degrading abilities, a classification into copiotrophic and oligotrophic was suggested (Fierer *et al.* 2007) using a similar concept like K- and r-strategists used for plants and animals. K-strategists are adapted to more stable environments. They grow slowly, reproduce rarely and cope with resource limitation and high competition. In contrast, r-strategists are adapted to unstable conditions and can take fast advantage of sudden favourable environmental conditions. They grow very fast, have a short generation time and reproduce quickly. On that note, microorganisms can be categorized into copiotrophic and oligotrophic classes (high and low substrate demand and proliferating times). Thus, in soil, *Acidobacteria* are oligotrophic whereas *Bacteroidetes* and *Betaproteobacteria* are considered as mostly copiotrophic bacteria as they were found worldwide in soils with low and high carbon content, respectively (Fierer *et al.* 2007).

To identify bacteria utilising a certain carbon source, stable isotope probing (SIP) of nucleic acids is a powerful and culture-independent method. Here, the substrate of interest is labelled with ^{13}C and added to the soil. Bacteria assimilating this substrate will incorporate the ^{13}C into their DNA or RNA. With isopycnic centrifugation and subsequent fractionation those labelled nucleotides can then be separated from those without labelling because of density dissimilarities (Neufeld *et al.* 2007, Whiteley *et al.* 2007).

SIP is often used to identify bacteria involved in degradation of certain substrates in soil. In some studies just one specific labelled compound was applied to identify the bacteria utilising them like acetate, glucose (Monard *et al.* 2008), phenol, caffeine and naphthalene (Padmanabhan *et al.* 2003), methanol (Lueders *et al.* 2004) or cellulose (Haichar *et al.* 2007). Amending labelled cellulose to different soil types, revealed high site specificity for fungi whereas some bacteria appear as universal degraders (*Burkholderiales ssp.*, *Sphingobacteriales*) and others were unique to only a few soils (*Myxococcales*, *Bacillales*, *Acidobacteria*) (Eichorst *et al.* 2012). The addition of a single compound substrates in high concentrations can cause excessive grow of dominating species and distort predictions about the distribution and the quantity of intrinsically involved bacteria under natural circumstances were nutrients are normally limited (Monard *et al.* 2008). For example, after excessive ^{13}C-phenol addition to soil, it was revealed that due to the input of this specific compound certain bacteria were highly enriched in comparison to the natural bacterial community at this site (DeRito *et al.* 2005).

Other studies used more natural substrates like rhizodeposits or litter from plants grown under $^{13}CO_2$ atmosphere. This ensures a natural composition and amount of the ^{13}C compounds added to the soil. Fresh wheat litter is mostly degraded by *Beta*- and

Gammaproteobacteria, especially *Massilia* spp., *Variovorax* spp. and *Pseudomonas* spp. (Bernard *et al.* 2007). At a later stage of composting, a shift towards *Actinobacteria* and *Alphaproteobacteria* (*Sphingomonas* spp.) was observed in studies without SIP (Bastian *et al.* 2009, Pascault *et al.* 2010). In the soil of a flooded anoxic rice field, rice straw is mainly degraded by *Clostridium* ssp. and *Methanosarcinaceae* (Shrestha *et al.* 2011).

In some studies, plants were exposed to $^{13}CO_2$ in order to introduce labelled rhizodeposits to the soil. By this, specific bacterial rhizodeposits users were identified for wheat, maize, rape and barrel clover, but also some common for all plant species like *Rhizobiales* and *Pseudomonas spp.* (Haichar *et al.* 2008). Vandenkoornhuyse et al. demonstrated that rhizoexudates are degraded almost simultaneously and already after five hours of labelling with $^{13}CO_2$, rhizodeposit consumers can be identified with SIP in intact grassland turfs (Vandenkoornhuyse *et al.* 2007). According to their study, mostly arbuscular mycorrhiza fungi (AMF), but also *Burkholderiales*, *Sphingomonadaceae* and *Acinetobacter* spp. were the primary organisms using rhizodeposits in the food web of grassland soil. Another study with labelled rhizodeposits reported altered carbon flow in a grassland soil food web with elevated atmospheric CO_2: Because of higher rhizodeposits release, not only AMF used rhizodeposits directly but also bacteria. Furthermore, changes in the community composition of fungi and bacteria can be observed (Drigo *et al.* 2010).

Not only primary consumers, but also trophic links to secondary microbes, fungi or even predators can be detected with ^{13}C-labelling and SIP by cross-feeding, especially in time resolved studies. In such an approach, the carbon flow through a food web starting from methane in a wetland rice soil was revealed. $^{13}CH_4$ was mainly taken up by methanotrophs which were than predated by *Myxobacteria*- and *Bdellovibrio*-related bacteria. Bacteria-preying protists like amoeba, flagellates and ciliates were also identified as secondary consumers (Murase *et al.* 2007). The primary consumers of rice root exudates were mainly *Azospirillum* spp., *Magnetospirillum ssp.* and *Burkholderiaceae* (Lu *et al.* 2006).

Also bacterial biomass itself can be used to track carbon flow in soil food webs in SIP studies. After adding ^{13}C-labelled biomass originating from *E. coli*, predation and decomposition from *Lysobacter* spp., *Myxococcales* and the *Bacteroidetes*, all assumed to be gliding bacteria, were observed (Lueders *et al.* 2006). Tracking the label from ^{13}C *Pseudomonas* spp. demonstrated that Collembola, identified as fungivore in preferential-food-experiments, rather diet on bacteria (Crotty *et al.* 2011).

Considering all these studies addressing various bacterial involvements in soil food webs, the “black box” is opening and the key players of the degradation of certain substrates are

more and more identified. But until now, the embedding of those identified bacterial food web members into the entire soil food web and their further links are only fragmentarily considered (McGuire *et al.* 2010) and comparison of degraders of different natural substrates in the same soil habitat are missing. Furthermore it is not clear, whether bacterial food web members are subject to temporal or spatial heterogeneities which are known to affect the composition of the overall bacterial community in soils.

1.2.4. Distribution of bacteria in soils

A global investigation of the bacterial communities in soils from different biomes all over the world revealed, that at least always the same phyla (*Proteobacteria, Actinobacteria, Acidobacteria* and *Bacteroidetes*) are dominant in soil but with varying frequencies. (Fierer *et al.* 2009). On more specific taxonomical level, however, soils comprise very diverse and variable bacterial communities.

One gram of soil contains up to 10^{10} bacterial cells (Trevors 2010) and around 52,000 species (Roesch *et al.* 2007) and is composed of many pores, particles and soil aggregates that form spatially divided habitats for bacteria (Grundmann 2004, Nunan *et al.* 2003). As bacterial size ranges mostly only around 1 µm (Zinder *et al.* 2006), the influencing surrounding is very small allowing for distinct niche forming. This already indicates, that bacterial distribution in soil is increasingly heterogeneous all the smaller the considered habitat becomes.

Nevertheless, differences in bacterial communities at bigger scales can be observed as well and a variety of differentiators have been reported. Most relevant effects were observed for soil depth, soil compartment (rhizosphere, bulk soil), season and cultivation as elucidated in detail in this paragraph.

Above all, bacterial communities change with soil depth. Not only biomass and cell numbers decrease with depth (LaMontagne *et al.* 2003, Shamir *et al.* 2007) but also diversity and richness (Fierer *et al.* 2003, Will *et al.* 2010). Besides this numerical reduction, altered function was observed, and bacteria from deeper horizons possess lower ability to use easily degradable substrates compared to their upper equivalents (Griffiths *et al.* 2003). This functional shift is accompanied by an altered composition of the bacterial community with depth. Especially *Actinobacteria, Bacteroidetes, Alpha- Beta-* and *Gammaproteobacteria* prefer the top soil whereas *Acidobacteria* are more frequent in lower soil layers of grassland soils (Fig. 3, Eilers et al. 2012, Will et al. 2010).

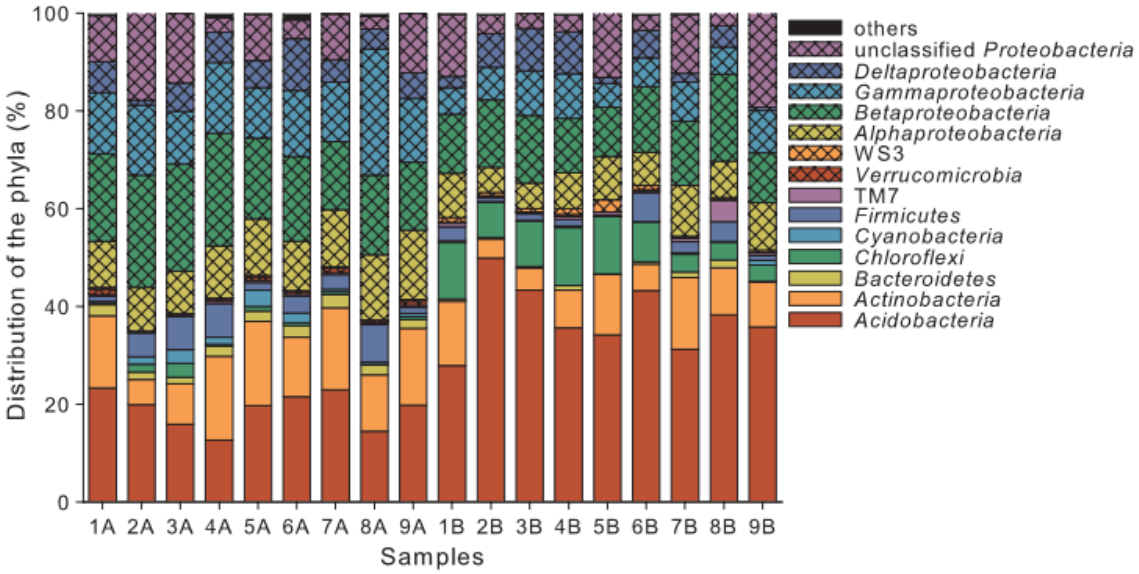

Fig. 3: Distribution of bacterial phyla for different grassland sites (1-9) within the A and B horizons (taken from Will *et al.* 2010).

Different bacterial community patterns can be observed not only at different depths but also for the same horizons but with altered soil properties (Berg *et al.* 2009, Fierer *et al.* 2009). Three different soil types equally cropped with strawberry and oilseed rape exhibited different community structures (Costa *et al.* 2006) and also bacterial species degrading cellulose vary with soil type (Eichorst *et al.* 2012).

Especially pH, carbon content, soil moisture and electrical conductivity, an indication for soil mineral content influence the community composition of soil bacteria (Gelsomino *et al.* 2011). Moreover, nitrogen content and availability has a considerable influence, too (Will *et al.* 2010).

Additional to the spatial heterogeneity, soil bacterial communities are subject to temporal shifts as well, especially in regions exposed to seasonal weather changes. For soils in the northern temperate climate zone with four distinct seasons, increase in top soil bacterial biomass (Dornbush *et al.* 2008) and an enhanced ability to use labile carbon compounds (Griffiths *et al.* 2003) can be observed in early summer. Distinct community shifts due to sampling time was observed in a maize monoculture (Spedding *et al.* 2004) and in the soil of a restored grassland (Habekost *et al.* 2008). For deserts with semi-arid climate, changes are observed for the dry and wet seasons (Shamir *et al.* 2007). In another grassland study seasonal changes even masked effects of controlled rain intensity and frequency rates (Cruz-Martinez *et al.* 2009). However, in comparison with depth, seasonal changes have less influence on the community composition (Bausenwein *et al.* 2008).

Various aboveground influences like land use or plant species can have effects on belowground bacteria, too. For examples, restoration of an arable field to natural grassland leads to a significant change in the bacterial and archeal community structure for early succession state (Kuramae *et al.* 2011) Also, type and amount of fertilizer influence bacterial communities, as important soil properties like carbon content and pH were changed upon amendment (Toljander *et al.* 2008). Furthermore, the different types of land uses of forest, grassland and arable field influence the bacterial community (Wallenius *et al.* 2011) but physico-chemical soil properties and depth are more important (Gelsomino *et al.* 2011). Dominating effects of soil properties were also observed in a study about effects of crop rotation (Suzuki *et al.* 2012).

In addition to the strong impact of soil properties, also plants can influence the bacterial community in soils and certain bacterial species have been associated with specific plant species regardless of soil types (Costa *et al.* 2006). Certainly, this is most prominent at the rhizosphere but on top of this, plants can also influence directly (exoenzyme discretion) and indirectly (priming effect) the bulk soil community (Haichar *et al.* 2008).

However, the rhizosphere is the soil compartment where plants have the strongest influence and it is a special habitat for soil bacteria. Soils are mostly carbon restricting (Will *et al.* 2010) but roots release a variety of organic substrates by root exudates, mucilage and dead cells of the root cap. Furthermore, lysates can leak from feeding damages caused by grazing soil fauna (Dennis *et al.* 2010).

Especially root exudates are known to be a way for plants to directly alter the chemical conditions in the root environment and shape the microbial community composition in favour of pathogen antagonists and plant growth promoting rhizobacteria (PGPR; Hartmann *et al.* 2009a). Root exudates are released actively into the soil and differ by plant species and growth state of the plant (Doornbos *et al.* 2012, Hartmann *et al.* 2009a). They consist of a mixture of organic compounds: mostly sugars, polysaccharides, amino acids, and organic acids but also sterols, phenols, enzymes, proteins, plant growth regulators and secondary metabolites (Badri *et al.* 2009, Carvalhais *et al.* 2011).

The bacteria in the rhizosphere origin from the surrounding bulk soil but they are selected particularly by their aptitude to adapt to rhizosphere conditions. Well adapted species from *Beta-, Gamma-, Alphaproteobacteria* (especially *Burkholderiales ssp.* and *Pseudomonas ssp.)* and *Actinobacteria* are frequent in the rhizosphere of many plant species but also *Bacteroidetes, Acidobacteria* and *Verrucomicrobia* are typical inhabitants (Buée *et al.*

2009). They are selected by certain abilities like chemotaxis, mobility and rhizodeposit utilisation and a copiotrophic life style is promoted in this habitat (Fig. 4, Berg *et al.* 2009).

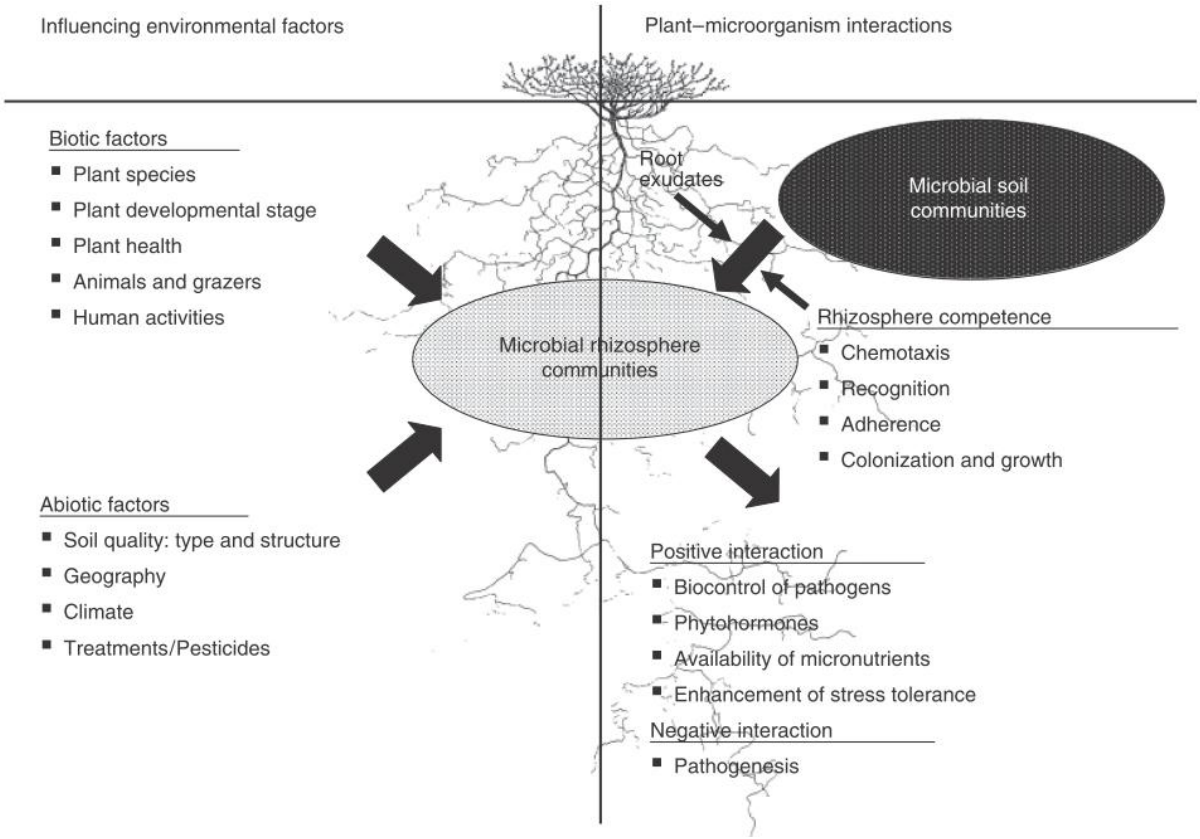

Fig. 4: Key factors influencing rhizosphere microbial communities and a conceptual model how rhizosphere bacteria are selected from soil (taken from Berg *et al.* 2009).

Furthermore, plants can select directly for pathogen antagonists by the composition of their root exudates (Berendsen *et al.* 2012). Many bacteria like *Arthrobacter* spp., *Pseudomonas* spp., *Streptomyces* spp. and *Xanthomonas* spp. are known to keep pathogens under control by mere competition and production of antibiotics (Doornbos *et al.* 2012). Another supported bacterial group are plant growth promoting rhizobacteria (PGPR) like symbiotic (e.g. *Rhizobium* spp., *Bradyrhizobium* spp.) and free-living nitrogen fixers (e.g. *Azospirillum* spp., *Pseudomonas* spp.), phosphorus-solubilizing bacteria (e.g. *Pseudomonas* spp., *Bacillus* spp. and *Rhizobium* spp.) and bacteria producing plant growth hormones like auxins and cytokins (e.g. *Azotobacter* spp., *Rhizobium* spp., *Pseudomonas* spp.) (Hayat *et al.* 2010).

There is extensive evidence, that plants have essential influence on bacterial communities. However, the substrate induced rhizosphere effects cannot just be assigned directly to root deposits, as they are only released near the root tips and are rapidly converted by the

microorganisms. That constrains the direct influence of plant derived substrates to a small temporal and spatial range (Dennis *et al.* 2010).

Up to now, we already have a profound understanding how bacterial communities are affected by soil properties and other environmental influences but little is known in respect of the bacteria relevant for food webs. This aspect demands attention especially considering the fact that a large part of soil bacteria are inactive or dormant. On average, more than 80 % of all bacterial cells can be inactive in soils with a range between 60 % and 96 % depending on substrate availability (Dornbush *et al.* 2008, Lennon *et al.* 2011).

Therefore, it is important to not only investigate and describe overall bacterial communities, but to account for functionality and identify bacterial food web members and their distribution in soil as a function of spatio-temporal controls.

1.3. Transport of bacteria and carbon into subsoil

Although transport of SOM from top soil to deeper soil layers is an important aspect of global carbon cycling (Rumpel *et al.* 2011), it is mostly neglected in studies about soil food webs (Dungait *et al.* 2012). Presumably, transported SOM has not been considered in food webs so far because it has no active part in trophic interactions.

Generally, SOM in subsoils is known to be very stable. Old subsoil SOM is not easily degraded as it has low energy content and unfavourable C/N ratios. Furthermore, SOM in subsoils is often spatial inaccessible for microorganisms (Dungait *et al.* 2012). Transported fresh carbon from top soil, however, is energy-rich and provides even enough energy for bacteria to degrade recalcitrant subsoil SOM (Fontaine *et al.* 2007). This mechanism is known as ‘priming effect’ (Kuzyakov 2010). Vertical carbon fluxes in soil can be considerable (Giardina *et al.* 2005) and represent a very important supply of fresh carbon to deeper soil layers.

The mobile organic matter pool in soils comprises mostly dissolved but also colloidal organic carbon including biocolloids like bacteria, fungi and their fragments as well as viruses (Totsche *et al.* 2007). The translocation of colloids and particles, frequently along preferential flow pathways including biopores can mediate fast and considerable mass transfer into deeper zones. Such transported bacteria could be an important source of biomass for subsoils and possibly affect the soil food web in two ways: Either as mere carbon source or they may actively contribute to carbon turnover (Jaesche *et al.* 2006) especially as active bacteria are more easily mobilised than inactive ones because of lower surface hydrophobicity (Gargiulo *et al.* 2007).

The general understanding of the physical factors controlling vertical carbon transport through soil has improved over the last years (Bolan *et al.* 2011, Kalbitz *et al.* 2008). Already now, there is a basic grasp of bacterial transport mechanisms in soils, mostly derived from studies with focus on the transport of potential pathogens to groundwater (Bradford *et al.* 2013). Important factors inhibiting bacterial mobilisation are retention at air-water and soil-water interfaces, attachment, straining but also active adhesion (Sen 2011). Soil bacteria can move actively in soils guided by chemotaxis (Sen 2011) or may be mobilised and transported passively by water flow (Unc *et al.* 2004), nematodes (Knox *et al.* 2004), growing roots (Feeney *et al.* 2006), or along fungal mycelia (Furuno *et al.* 2012). However, the highest fluxes of bacterial pathogens transported from aboveground to deeper soil layers occur after weather events producing abundant seepage water, such as long-lasting precipitation, flooding or snow melt. Especially the detachment of top soil microbes by rain and snowmelt water with low-ionic strength is assumed to contribute to this mobilisation (Aislabie *et al.* 2011). Once mobilised, transport is assumed to be controlled mainly by the flow of seepage water along macropores, e.g., earth worm burrows, root channels and desiccation cracks (Natsch *et al.* 1996). Troxler and colleagues observed transport of added bacteria from top soil down to depths of ~2.5 m only after heavy rainfalls (Troxler *et al.* 1998). The main route of transport was flow along macro pores, which was confirmed also by other more recent studies (Bech *et al.* 2011, Jiang *et al.* 2010).

Practically all studies on bacterial transport in soils and porous media have used only one or a few artificially added bacterial species, and did not address mobilised natural bacterial communities. So, while many factors influencing the transport of carbon and bacteria over depth have been investigated, an understanding of the origin and nature of transported soil bacteria, their contribution to carbon fluxes to deeper zones as well as their role in soil food webs is currently lacking.

1.4. Outline of this PhD thesis

This PhD project is part of the DFG-funded Research Unit FOR 918 ("Carbon flow in belowground food webs assessed by isotope tracers") which has been initiated to generate a better understanding of carbon flow through all trophic levels and organisms of a belowground food web. For this, an agricultural soil and fresh plant-derived carbon inputs are used as model system. The aim of the Research Unit is to quantify the carbon fluxes in the food web and to correlate them to the different organisms involved. Furthermore, the

trophic links between these organisms are to be specified and quantified, and eventually a comprehensive model of carbon flow in this belowground food web will be constructed. In a concerted and interdisciplinary large-scale study, the full range of involved organisms from microorganisms to meso- and macrofauna has been considered.

Maize (a C_4-plant) was grown on a soil, which had been cultivated only with C_3-plants for more than a decade. As there are differences in the $^{12}C/^{13}C$ ratio for C_3- and C_4-carbon fixation, fresh carbon from the maize can be detected not only in the soil but also in organisms consuming fresh plant-derived substrates, as well as in their predators and decomposers. Because of different cultivation treatments set up by the Research Unit, plant-based substrate inputs were distinguished into carbon from litter added after harvest and carbon from rhizodeposits.

Within that Research Unit, I specifically addressed with my PhD thesis the bacteria involved in channelling plant-derived C into the soil food web, as well as secondary consumers. Here, I wanted to identify bacteria consuming rhizodeposits, detritus substrates and bacterial biomass and localise their distribution in an agricultural field with regards to soil compartment (rhizosphere, bulk soil), depth and season. In a dedicated field-to-lab-to-field approach, stable isotope probing (SIP) of nucleic acids was employed in laboratory experiments to directly identify key bacteria relevant in the food web and their association with distinct substrates.

With regard to the state-of-the-art considerations above, I hypothesise that:

1. **Bacterial populations are underrepresented in current concepts of soil food webs.** Soil bacterial populations are not restricted to a single “black box”, but contribute distinctly to the turnover of both labile and recalcitrant substrates and occupy more than just one trophic niche in soil food webs.

2. **Key bacteria in soil food webs are not evenly distributed in space and time.** Given the heterogeneities in the availability and quality of carbon substrates in soils, bacteria utilising fresh plant derived carbon inputs are expected to be more abundant in the rhizosphere and top soil especially in summer, whereas bacteria using litter should be more relevant in bulk soil and winter.

3. **Distinct soil bacterial populations are selectively mobilised** from the top soil by seepage water and **transported bacteria contribute noticeably to net carbon flux over depth**.

To verify these hypotheses and to open the “black box” of soil bacteria by identifying key soil food web members, a strategy was designed for this thesis with four experimental approaches:

1. Investigation of the spatial heterogeneity and seasonal dynamics of the overall soil bacterial community at the exploratory field of the Research Unit
2. Identification of key bacteria involved in the channelling of carbon from plants into the belowground food web by stable isotope probing in microcosm experiments
3. Determination of the spatial distribution and abundance of those identified food web bacteria in the exploratory field
4. Elucidating the role of mobile microbial community members in the event-driven export of top soil carbon to deeper soil layers

All experiments of the Research Unit and consequently of this PhD project were conducted with soil of an experimental field near Göttingen, Germany. For the field campaigns soil samples were directly taken from the field while for the lab experiments the same soil was used in microcosms. A graphical overview of the experimental design and the different approaches are given in Fig. 5. Further down, the experiments are presented in detail.
All work that was done in cooperation and the respective contributions of other members of the Research Unit to my project will be explicitly indicated. If not stated otherwise, experiments and analyses were conducted by the author.

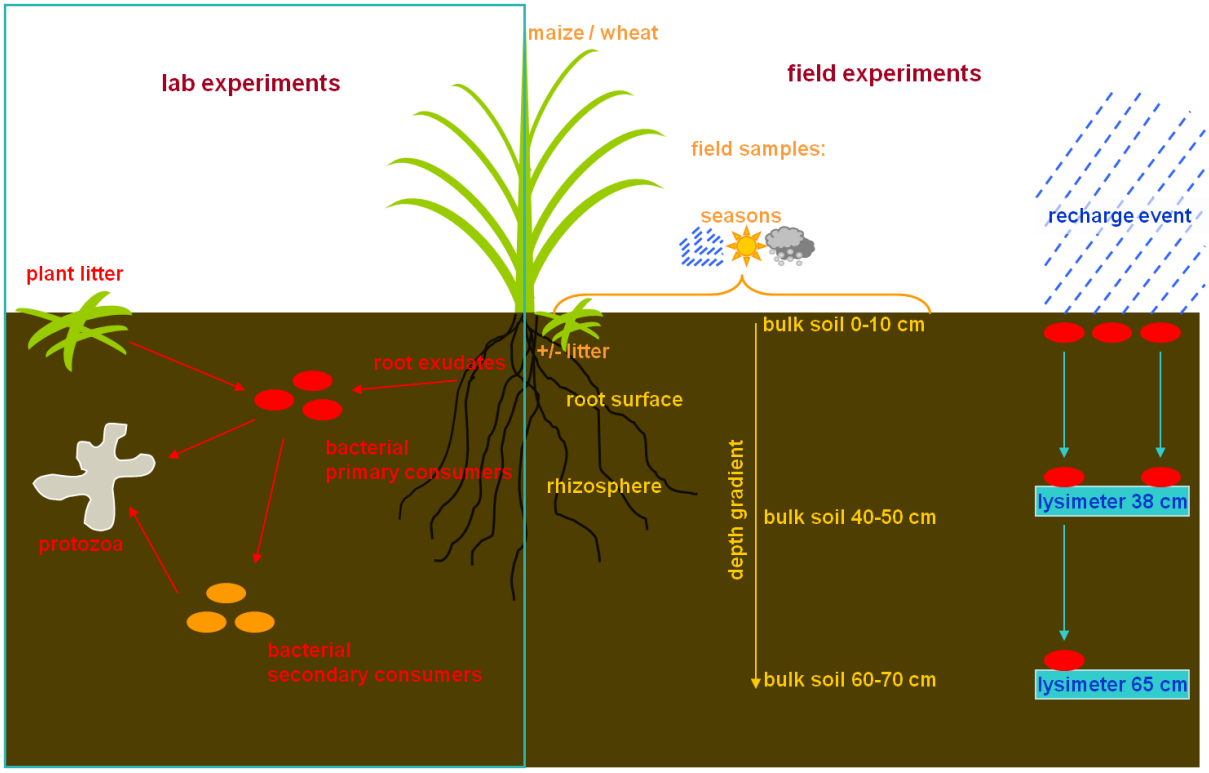

Fig. 5: Graphical overview of the lab and field work conducted within this thesis.

1.4.1. Field sampling and investigation of the bacterial community distribution *in situ*

Together with the members of the Research Unit, I took triplicate samples from three different depths on four time points during the year to cover a wide range of the natural variation of this habitat. The samples originated from the top soil (0-10 cm), the subsoil below the plough layer (40-50 cm) and from the root free zone (60-70 cm). In accordance with seasonal changes during the year, samples were taken right before planting (May), at highest exudation rate (July), right before harvest and input of dead plant material (September) and in winter (December), when relocation of DOM (dissolved organic matter) and MOPS (mobile organic particulate substances) is highest. To be able to discriminate the carbon from different substrate sources, four cultivation treatments were established at different field plots: cultivation with either wheat or maize plants each with or without amendment of maize litter.

From those soil samples, I analysed the bacterial community composition with 16S RNA gene terminal restriction fragment length polymorphism (T-RFLP) fingerprinting (and partially 16S RNA fingerprinting) to get insight into the distribution and diversity of the bacteria in the soil dependent on depth, season and cultivation treatment. Furthermore, I quantified the overall bacterial 16S RNA gene abundance by qPCR and I identified the most abundant bacteria by amplicon pyrosequencing for chosen soil samples.

1.4.2. Identification of key bacterial food wed members by stable isotope probing

Rhizodeposit and detritussphere SIP

To identify the bacteria involved in the direct turnover of plant-derived carbon compounds such as rhizodeposits (labile) and litter (more recalcitrant), I applied stable isotope probing (SIP). Labelling of bacterial primary consumers of rhizodeposits was done in a laboratory microcosm experiment together with Maike Hünninghaus and Robert Koller from Michael Bonkowski's group (Department of Terrestrial Ecology, University of Cologne), Microorganisms consuming glucose, cellulose, shoot and root litter were labelled by Susanne Kramer at Ellen Kandeler's group in Hohenheim (Institute of Soil Science and Land Evaluation, University of Hohenheim). Subsequently, I analysed the RNA of bacteria of these experiments by SIP, T-RFLP fingerprints and amplicon pyrosequencing and evaluated the resulting data.

Biomass SIP

In a third microcosm experiment, I labelled putative root exudate utilising bacteria by artificial model ^{13}C exudate compounds to identify intra-bacterial secondary trophic links. I identified bacterial secondary consumers again by stable isotope probing, T-RFLP fingerprinting and amplicon pyrosequencing.

1.4.3. Back to the field: identified bacterial food web members in their natural habitat

Beside the identification of key bacteria involved in plant-derived carbon flow, I estimated the amount of these taxa within the total microbial community with regard to spatial and temporal changes. I will provide this field-based quantitative population data of specific key food web bacteria for the food web modelling in this Research Unit (Ulrich Brose, University of Göttingen). The share of key bacteria on the whole microbial biomass was assessed depth and time depending by use of qPCR and amplicon pyrosequencing data as well as microbial biomass (measured by Susanne Kramer, University of Hohenheim).

1.4.4. Mobilisation and transport of soil bacteria by seepage water

Zero-tension lysimeters for seepage water collection were installed at the field and sampled by members of the group of Kai-Uwe Totsche (Institute of Geosciences, University of Jena). To determine if bacteria were mobilised and transported selectively to deeper zones after snow melt and rain, fingerprinting analysis and amplicon sequencing was performed

from the lysimeter water and corresponding soil samples. Furthermore, qPCR data, microbial biomass (Susanne Kramer) and organic carbon content measures (Andreas Schmalwasser, Institute of Geosciences, University of Jena) were used to estimate how important bacteria are for the carbon input into deeper soil zones and to what extend bacteria contribute to this process.

Altogether, the work conducted in this thesis contributes to a better understanding of the functioning of distinct bacterial populations in soil food webs and carbon cycling in soils.

2. Materials and Methods

2.1. Site, soil and sampling

All experiments of this thesis and of the other studies of the research group were conducted with or within the soil of the same exploratory field. This agricultural field is located on a terrace plain of the river Leine, north-north-west of the City of Göttingen in Niedersachsen, Germany (Fig. 6). Planning, set-up and management of the field was chiefly done by the group of Stefan Scheu (J.F. Blumenbach Institute of Zoology and Anthropology, University of Göttingen).

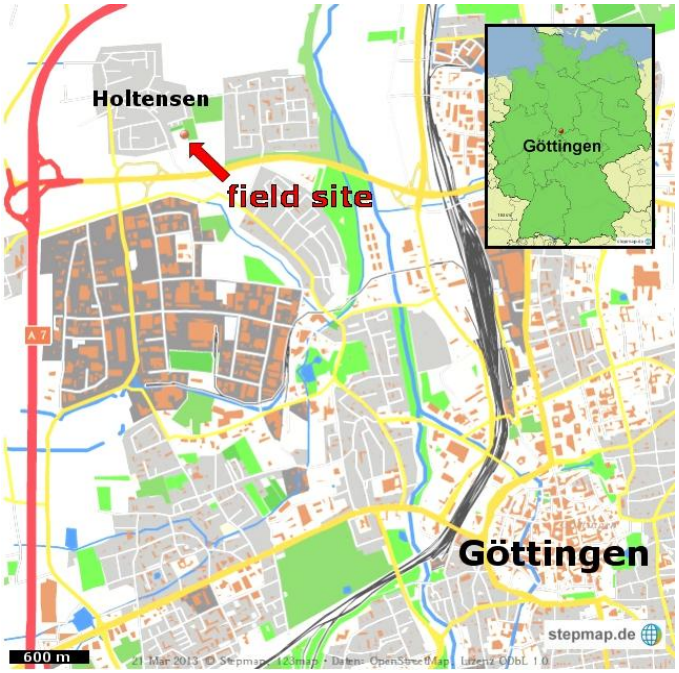

Fig. 6: Location of the field site (with friendly permission of stepmap.de).

2.1.1. Field characteristics

The local climate, with a mean annual temperature of 8.7 °C and mean annual precipitation of 645 mm, represents a temperate climate zone, affected by the transgression from the maritime Atlantic climate from the west to the continental climate to the east. The elevation of the plane is 155-160 m above sea level, striking towards north-west with a mean base slope of approximately 2 %. According to the International Union of Soil Sciences (IUSS Working Group WRB 2007), the dominant soil types are Cambisols (Braunerden, KA5 2005), Luvisols (Parabraunerden, KA5 2005) and stagnic Luvisols (Pseudogley, KA5 2005). However, long agricultural use has severely affected the build-up

of the soil profiles. The albic horizon typically found for these soils can no longer be detected in the field due to centuries of intensive tillage. In general, two plough layers (0.2 m and 0.3 m below surface) can be detected, with strong compaction below the second plough layer in particular. This is especially evident in the relatively high bulk density (1.6 g cm^{-3}) in and below the second plough layer (Kramer *et al.* 2012). The border between A and B horizons vary along the field. In Fig. 7, the border of the A horizon is depicted for field segments of 15 m x 20 m.

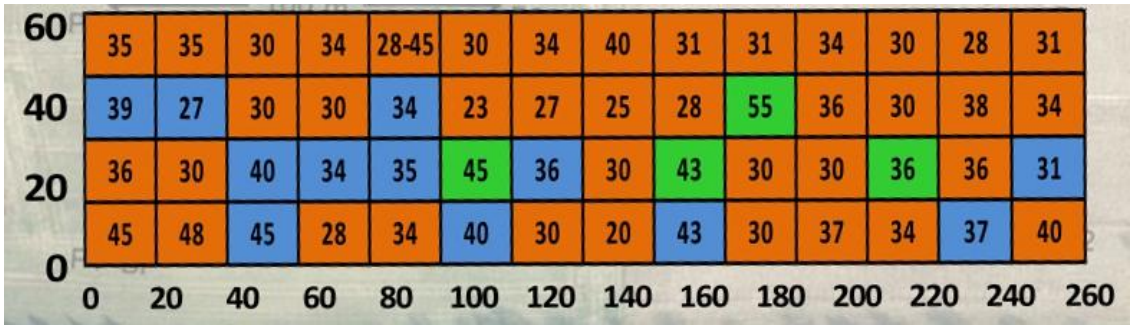

Fig. 7: Borders between A and B horizons in the field [cm]. Colours depict the soil type: orange: Luvisols, blue: stagnic Luvisols, green: Cambisols. Soil assessment was done by the group of K. Totsche (Univ. Jena) within the Research Units program.

2.1.2. Soil sampling

Sampling took place in May, July, September and December 2009 and July 2010. Sampling times were chosen to cover all important seasonal influences on the soil: before seeding, at highest root exudation during flowering, at harvest time and in winter when most fresh dissolved organic matter is released into soil. For lysimeter water sampling, January was chosen as it was the only time after lysimeter installation in that year with enough seepage water.

For the different cultivation treatments, the field was segmented into 20 plots (
Fig. 8).

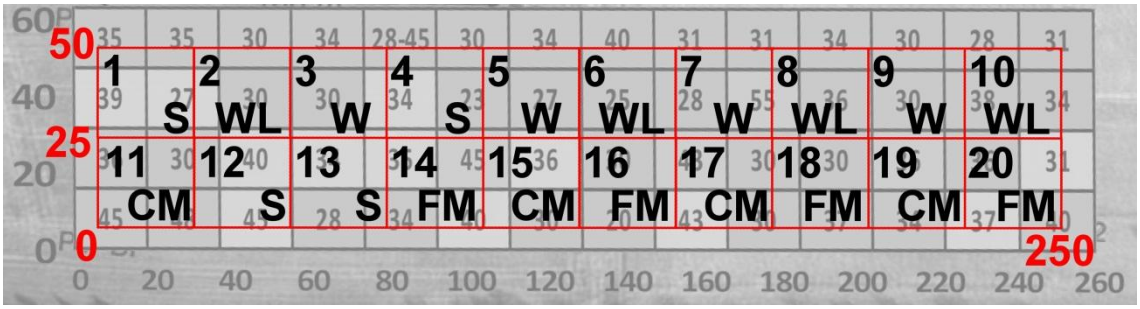

Fig. 8: Segmentation of the field site with plot numbers and treatment abbreviations. Plot size is 25 m^2 and each treatment has four replicates. S: supplementary plots, WL: wheat with maize litter, W: wheat, CM: corn maize, FM fodder maize. Overlay of plot over soil type description in Fig. 2. Red numbers: distance in m.

The northern 10 plots were cultivated with wheat whereas the southern plots were cultivated with maize. This was done for carbon budgeting and assessment of soil fauna conducted by other Research Unit members (Johanna Pausch, Y. Kuzyakovs group, University of Göttingen, Nicole Scheunemann, S. Scheus group, University of Göttingen, Susanne Kramer, E.Kandelers group, University of Hoheheim and Anika Scharroba, L. Ruess' group, University of Berlin). They used carbon isotope ratios to determine soil and organismic carbon recently derived from new plant inputs. Therefore, a field site was selected that was only cultured with C_3 plants so far. C_4 plants like maize use different enzymes to fix carbon from CO_2 with a different incorporation ratio of ^{12}C and ^{13}C. Considering shifts of isotope composition along the food web levels, carbon compounds newly derived from the maize plants can be discriminated against those from older soil organic carbon. To track carbon inputs with different qualities (rhizodeposits and litter), four cultivation treatments were applied altogether: fodder maize (FM, without litter amendment), corn maize (CM, with litter except corncobs), wheat (W) and wheat with maize litter (WL) (

Fig. 8). 0.8 kg (dry weight) m^{-2} (equal to 0.35 kg C m^{-2}) Maize litter was added to every second plot (CM and WL) in autumn right after harvest. Otherwise, the field was treated with tillage and herbicides as in conventional agriculture (Kramer *et al.* 2012).

Soil samples were taken as composite samples. 8 to 10 single samples were taken via Pürkhauer coring (∅ 3 cm, length 70 cm) and the cores were then divided into depth sections of 10 cm. The soil of the corresponding sections of the soil cores from each plot where then pooled. Then, a subsample was obtained from this pooled sample. The sample pooling ensured that each group of the Research Unit analysed the same soil. For this

study, three plots of each treatment were sampled as replicates in three depths: top soil (0 - 10 cm), below the plough layer (40-50 cm) and deeper soil at 60-70 cm.

Beside the bulk soil samples, root balls from maize stalks were also sampled. All soil and root samples were frozen within 6 h at -20°C until further use. Soil samples were sieved before DNA extraction and occasional root fragments were removed. To obtain rhizosphere and root surface samples, the root balls were thawed and then manually shaken until all readily detachable soil fell off. Root subsamples were then cut off with a sterile scalpel, and washed twice by shaking with 25 ml 1x PBS buffer in a 50 ml centrifuge tube. After washing, buffer and suspended solids were decanted into a fresh tube and the rhizosphere soil was collected by centrifugation (3345 g, 30 min). The remaining washed roots were chopped into bits of 2 cm and were also used for nucleic acid extraction.

2.1.3. Lysimeter sampling

The group of Mr. Totsche from University of Jena installed tension controlled lysimeters (KL2-100, UMS, Munich, Germany) directly below the plough horizon in approximately 35 cm depth and below the main rooted zone in 65 cm depth. The lysimeters were packed with undisturbed soil monoliths that were placed on top of a porous plate (pore size of 10 µm, SIC275, UMS, Munich, Germany). The lysimeter samples for this thesis were taken in January 2011. The sampling of lysimeters always implies a certain contamination risk, as biofilms from the installation or from tubes can distort the composition of seepage water biota. We minimized this risk by using sterilised sampling bottles, regularly inspecting the tubes for biofilms, and by maintaining a minimal retention time of fresh water samples in the lysimeters of only 24 h. Immediately after sampling, the water for the bacterial analyses was filtrated (0.2 µm Corning, New York, USA), and filters were then frozen at -20 °C until further processing.

2.2. Nucleotide extraction

2.2.1. DNA and RNA co-extraction

The nucleotide extraction protocol was modified from Lueders et al. (2004) and 0.4 g (wet weight) of soil (bulk and rhizosphere), 0.5 g of roots or half of a water filter were mixed with 750 ml $NaPO_4$ (pH 8), 250 ml TNS buffer (500 mM Tris, 100 mM NaCl, 10 % (w/v) sodium dodecyl sulphate) (pH 8) and 250 ml phenol-chloroform-isoamylalcohol (25:24:1, pH 8). The cells were lysed by bead beating with 0.2 ml of a 1:1 (v/v) mixture of 0.1 mm

zirconia/silica beads and 0.7 mm zirconia beads (Biospec Products Inc., Bartlesville, Okla., USA). Bead beating was done in a cell disruptor (FastPrep®-24, MP Biomedicals LLC, Santa Ana, Calif., USA) for 45 s at a setting of 6.5 ms^{-1}. After centrifugation (10 min, 4 °C, 15,000 rcf) the liquid phase with the nucleotides was mixed with an equal volume of phenol-chloroform-isoamylalcohol (25:24:1, pH 8) and the phases were separated again by centrifugation (4 min, 4 °C, 20,000 rcf). To remove the phenol, the liquid phase was extracted and transferred to a Phase Lock Gel Heavy 2 ml vial (5 Prime, Hamburg, Germany) and mixed with an equal volume of chloroform-isoamylalcohol (24:1) followed by centrifugation (4 min, 4 °C, 20,000 rcf). Afterwards, the DNA from the extracted liquid phase was precipitated by mixing with two volumes of PEG solution (30 % (w/v) polyethylene glycol 6000, 1.6 mM NaCl) and centrifugation for 2 h at 4 °C and 20,000 rcf. The DNA pellet was than washed with ice cold 70 % (v/v) ethanol, air dried and dissolved in 80 µl Buffer EB (Qiagen GmbH, Hilden, Germany). DNA from the two filter halves were dissolved in the same 80 µl of EB Buffer. Samples were kept on ice between extraction steps.

2.2.2. Purification and gel electrophoresis

For purification and elimination of humic substances, silica gel columns were used from the DyeEx 2.0 Spin Kit (Qiagen GmbH, Hilden, Germany) which had been equilibrated twice with 80 µl Buffer EB prior to that.

Nucleotide purity and integrity was inspected by gel electrophoresis. 5 µl of DNA/RNA extract were mixed with 2µl of loading dye (Thermo Fisher, Waltham, Mas., USA) and applied to a 1.5 % agarose gel. Gel electrophoresis was done with 1xTAE buffer (242 g Tris base, 57, 1 ml glacial acetic acid, 100ml 500mM EDTA pH8, ad 1l DI water) at 90 V and 150 I for 45 minutes. Afterwards, the gel was stained for 10 minutes with 3 % GelRed (Biotium, Hayward, Calif., USA) and DNA/RNA bands were visualised under UV light. Only if the bands of 16S and 23S RNA were clearly visible and contamination by humic acid was negligible, DNA/RNA was used for further analysis.

2.2.3. RNA extraction

To remove DNA from the DNA/RNA extracts, RQ1 RNAse-free DNAse (DNAse I) was used (Promega, Fitchbury, Wis., USA). 100 µl DNA/RNA were mixed with 50 µl DNAse I (2 u/µl RNA), 20µl buffer and 30 µl H_2O and incubated for 2h at 37°C. Then, RNA was extracted as described above by mixing with 100 ml phenol-chloroform-

isoamylalcohol (25:24:1, pH 8), subsequent centrifugation (10 min, 4 °C, 20,000 rcf), mixing with 100 ml chloroform-isoamylalcohol (24:1) and centrifugation in a Phase Lock Gel Heavy 2 ml vial (5Prime, Hamburg, Germany) precipitation with 200 ml PEG solution (30 % (w/v) polyethylene glycol 6000, 1.6 mM NaCl) and centrifugation for 2 h at 4 °C and 20,000 rcf, pellet washing with ice cold 70 % (v/v) ethanol, air drying and pellet dissolution in 50 µl Buffer EB (Qiagen GmbH, Hilden, Germany).

Purity, integrity and quantity were analysed accordingly to DNA/RNA extracts with gel electrophoresis. RNA quantity was measured with a NanoDrop® ND-1000 (Thermo Fisher, Waltham, Mas., USA)

2.3. Terminal restriction fragment length polymorphism (T-RFLP)

T-RFLP fingerprinting was done largely as reported by Winderl et al. (2008) and the detailed description of individual steps are described below.

2.3.1. Polymerase chain reaction (PCR)

The bacterial and partially the eukaryotic community in the soil, root and lysimeter water samples were analysed by 16S rRNA gene-targeted terminal restriction fragment length polymorphism (T-RFLP) fingerprinting with primers specified in Table 1. Forward primers were always labelled with FAM (3′,6′-Dihydroxy-1-oxospiro[2-benzofuran- 3,9′-xanthen]-5-carbonsäure) for later detection of restriction fragments.

Table 1: Sequences of the primers used in this work (Euringer *et al.* 2008, Pilloni *et al.* 2011)

primer	sequence
Ba27f	5’-AGA GTT TGA TCM TGG CTC AG-3’
Ba907r	5’-CCG TCA ATT CCT TTG AGT TT-3’
Euk20f	5’-TGC CAG TAG TCA TAT GCT TGT-3’
Euk519r	5’-ACC AGA CTT GYC CTC CAA T-3’

Prior to PCR, DNA was diluted 100 fold for soil samples from 0-10 cm, 40-50 cm and the rhizosphere, 10 fold for soil from 60-70 cm and for the root surface. DNA from lysimeter samples was used undiluted.

One PCR mix of 50 µl included 5 µl of 10 x PCR buffer, 3 µl of 1.5 mM $MgCl_2$,0.5 µl of 0.1 mM dNTPs, 0.25 µl (5u/µl) of recombinant Taq polymerase (all from Thermo Fisher, Waltham, Mas., USA), 0.5 µl of 0.2 µg μl^{-1} bovine serum albumin (BSA) (Roche,

Penzberg, Germany), 0.3 µl of each primer (50 µM) (Biomers, Ulm, Germany) and 1 µL of diluted template DNA. For amplification after initial denaturation (94 °C, 5 min) 25 cycles of denaturation (94 °C, 30 s), annealing (52 °C, 30 s) and elongation (70 °C, 60 s) were performed in a Mastercycler ep gradient (Eppendorf, Hamburg, Germany).

2.3.2. Reverse transcription polymerase chain reaction (RT-PCR)

The AccessQuick™ RT-PCR System (Promega, Fitchbury, Wis., USA) with the reverse transcriptase originating from avian myeloblastosis virus (AMV) was used for 16S rRNA. 2 µl of RNA were mixed with 0.3 µl of 50 mM forward and revers primers, 0.5 µl bovine serum albumin (20 µg μl^{-1}), 0.8 µl AMV reverse transcriptase (RT), 25 µl 2x Master Mix from the Kit and 21.1 µl RNA grade water. Reverse transcription was done at 45 °C for 30 minutes and subsequent PCR cycling was done as for normal PCR but with 68 °C for elongation instead of 70 °C. RNA-free and RT-free controls were added to each PCR run, to ensure RNA purity.

The AccessQuick™ RT-PCR Kit was not suitable for eukaryotic 18S rRNA amplification as it produced unspecific amplicons. Therefore, the Brilliant III Ultra-Fast SYBR®Green QRT-PCR Master Mix (Agilent Technologies, Santa Clara, USA) was used with a reverse transcriptase originating from maloney virus. Here, reverse transcription took 20 min at 45 °C and PCR cycling was done as for PCR stated above.

After confirmation of accurate amplification with gel electrophoresis (no product for negative controls, sufficient product at right ladder position for samples) the PCR products were purified with PCR Extract Mini Kit (5Prime, Hamburg, Germany) to remove PCR reagents, primers and nucleotide fragments below 50 bp. DNA amount of purified amplicons were measured by UV-photometry with a NanoDrop® ND-1000 Spectrophotometer (Thermo Fisher, Waltham, Mas., USA)

2.3.3. T-RFLP fragment analysis

Restriction was done in 10 µl with 80 ng DNA, 0.3 µl restriction enzymes and 1 µl buffer (all Thermo Fisher, Waltham, Mas., USA) for 2 h at 37 °C. Restriction enzyme was MspI for bacteria and Bsh1236I for eukaryotes. Afterwards, fragments were desalted with DyeEx 2.0 Spin Kit (QIAGEN GmbH, Hilden, Germany), mixed with high definition formamide which contained 6-carboxy-X-rhodamine-labelled MapMarker 1000 ladder (BioVentures, Murfeesboro, Tenn., USA) in 1:400 dilution. Fragments were denatured at 95 °C for 5 min and stored in the fridge (4 °C) until fragment analysis.

Automated fragment electrophoresis was performed at the genome analysis centre, a core facility at the Helmholtz Zentrum München. The fragments were separated by capillary electrophoresis on an ABI 3730 DNA analyser (Applied Biosystems, Darmstadt, Germany). Electrophoresis was performed with POP-7 polymer in a 50 cm capillary array under the following conditions: 10 s injection time, 2 kV injection voltage, 7 kV run voltage, 66 °C run temperature and 63 min analysis time.

The electropherograms were evaluated with the Gene Mapper 5.1 software (Applied Biosystems, Darmstadt, Germany) and T-RFLP data was analysed with the online T-RF analysis software T-REX (Culman *et al.* 2009). Background noise filtering (Abdo *et al.* 2006) was on factor 1.2 for peak heights and the clustering threshold for aligning peaks across the samples was set to 1 using the default alignment method of T-Align (Smith *et al.* 2005). Relative T-RF abundance was inferred from peak heights. For reduction of data complexity, T-RFs that occurred in less than 5 % of the samples were excluded from further analysis.

2.4. Quantitative PCR (qPCR) and reverse transcription quantitative PCR (RT-qPCR)

To estimate bacterial numbers from the field, qPCR was done for 16S and 18S RNA genes with a Stratagene MX3000P qPCR cycler (Agilent, Santa Clara, Calif., USA). Gene copy numbers were measured in three dilutions with three triplicates each. Dilution factors for the soil of 0 - 10 cm, 50 - 70 cm and rhizosphere were $1x10^{-3}$, $1x10^{-4}$ and $1x10^{-5}$, for 60 - 70 cm and root surface $1x10^{-2}$, $1x10^{-3}$ and $1x10^{-4}$ and for the lysimeter water samples $1x10^{-1}$, $1x10^{-2}$ and $1x10^{-3}$.

Quantitative PCR was performed as PCR described above but with the fluorescence dyes SybrGreen for DNA detection and ROX (both Life Technologies, Carlsbad, Calif., USA) for equilibration. After 40 cycles (PCR cycling as for PCR) the quality of the PCR products was verified by melting curve analysis. As standard, full length 16S rRNA gene of *Azoarcus* sp. with known concentration was used in seven dilutions between $1x10^{7}$ and $1x10^{1}$ molecules per µl was used to convert measured threshold cycles to copy numbers.

For RNA from stable isotope probing (see below) RT-qPCR was done with undiluted RNA to see possible labelling and to find the optimum cycle number for downstream analyses. RT-qPCR was conducted in the same way as RT-PCR but with the fluorescence dyes as in qPCR.

As standard references, *in-vitro* transcribed RNA of cloned 16S rRNA gene fragments from a bacterium affiliated to *Desulfosporosinus* and a flagellate affiliated to *Bodo* were used in a serial dilution of 10^8 to 10^1 templates per µl.

2.5. Amplicon pyrosequencing

To identify the bacterial groups which were participating in the food web and exhibited the different T-RFs, total DNA and RNA of a selection of representative soil samples were sequenced with a 454 GS FLX pyrosequencer (Roche, Basel, Switzerland).

2.5.1. Amplicon Pyrotag PCR and RT-PCR

Barcoded amplicons were generated for forward and reverse reads with the same PCR and RT-PCR conditions as specified above. Primers were fused with A or B adapters (for forward and reverse discrimination) and with multiplex identifiers (MID), a small barcode sequence. Amplicon integrity was verified by gel electrophoresis and PCR products were purified with the Agencourt® AMPure® XP - PCR Purification Kit (BeckmanCoulter, Brea, USA) as specified by the manufacturer.

The PicoGreen® dsDNA quantitation assay (Life Technologies, Carlsbad, USA) was used to measure amplicon numbers according to the manufacturer's instructions but with a different standard curve (2.5, 2, 1.5, 1, 0.5, 0.1, 0.05 and 0 µg ml^{-1}). Samples were measured in three dilutions and each in triplicates with a Stratagene MX3000P qPCR cycler (Agilent, Santa Clara, Calif., USA).

Then, samples were diluted to 1x 10^9 molecules μl^{-1} with following equation:

Equation 1:

$$molecules\ \mu l^{-1} = \frac{sample\ conc\ [ng\ \mu l^{-1}] \times 6.022 \times 10^{23}}{656.6 \times 10^{9} \times amplicon\ length\ [bp]}.$$

13 or 26 samples were than pooled for amplicon sequencing with picoTiterPlates divided into eighth or quarters, respectively. Pooled amplicon libraries were than diluted to 1x 10^7 molecules μl^{-1}.

Subsequent emulsion PCR and sequencing was done at the Helmholtz Zentrum München core facility centre for 454 sequencing by Marion Engel or Brigitte Hai (coordinator: Michael Schloter, Terrestrial Ecogenetics, HMGU) and by Katrin Hörmann (T. Lueders group, Groundwater Ecology, HMGU). Emulsion PCR was done as recommended by the manufacturer in the emPCR Method Manual – Lib-A SV for GS FLX Titanium series

(Roche, Penzberg, Germany). To find best bead enrichment (8 %) for plate loading, emulsion titration was done before each run in three different concentrations. For emulsion PCR, capture beads were loaded with amplicons, amplification mix and emulsion oil were added. To ensure encapsulated PCR amplification for every bead, the emulsion was established by shaking with a tissue lyser and subsequent amplification was performed. Beads were washed and recovered and amplicons bearing beads were bound to magnetic beads which retained the DNA beads on a magnet. By this, beads without amplicons were removed with the surrounding buffer. Finally, sequencing primers for forward and revers reads were annealed to the amplicons.

2.5.2. Pyrosequencing

Sequencing itself was done with a 454 GS FLX pyrosequencer and Titanium chemicals (Roche). Amplicon bearing DNA beads were mixed with packing beats and filled into the picoTiterPlate (PTP) as the middle layer between two layers of enzyme beads. On top a layer with PPiase beads prevents interferences between wells. Then sequencing took place with several automated rounds of alternating addition of either dATP, dTTP, dGTP or dCTP and washing. Each successful incorporation of a nucleoside resulted in the release of PPi which was than coupled to adenosine 5´ phosphosulfate by ATP sulfurylase. ATP in turn was hydrolysed again by luciferase and a fluorescent signal was emitted indicating bit by bit the nucleotide sequence of the amplicon.

2.5.3. Data processing

Only the immediate processing of the amplicon library and the sequencing was done by others. Subsequently, I analysed the sequence data with a similar approach as reported recently (Pilloni *et al.* 2012b).

Sequences from pooled amplicon libraries were separated according to their sample and primer barcode and then sequencing data was trimmed with respect to its quality scores with the GreenGens trimming tool (DeSantis *et al.* 2006) and default settings (good quality threshold: 20, window size: 40, percentage: 90). Afterward, sequences below 250 bp were eliminated and forward and reverse reads were separated with BioEdit (Hall 1999). Contigs were generated by aligning of the quality trimmed forward and reverse reads with SEQMAN II software (DNAStar, Madison, USA) using an assembly thresholds of 98% sequence similarity in a 50 bp window. Only complete contigs with forward and reverse reads were used for further analysis. Both, contigs and not aligned but trimmed reads were

submitted to RDP classifier (Wang *et al.* 2007) for taxonomic classification with a threshold of 70 %.

In-silico T-RF were generated for the contigs with the software TRiFLe (Junier *et al.* 2008) to compare T-RF and sequencing data sets and to possibly designate phylogenetic groups to experimentally detected TRFs.

2.6. Set-ups of microcosm experiments

2.6.1. Rhizosphere SIP labelling

Maike Hünninghaus and Robert Koller (Department of Terrestrial Ecology, University of Cologne) planned this experiment and it was accomplished with my help in Cologne. I identified the bacteria participating in a rhizodeposit dependent food web with RNA-stable isotope probing, T-RFLP fingerprinting, amplicon sequencing and analysed the resulting data set.

The experiment was conducted in two acrylic glass chambers (l: 95 cm, w: 42 cm, h: 70 cm) for the ^{13}C labelling and the ^{12}C control, respectively. To simulate summer conditions, plants were exposed to light for 12 hours each day with 600 µmol PAR (photoactive radiation). Temperature was 28 °C by day and 18 °C at night when the chamber was opened.

Maize plants sprouted from corn on wet cellulose tissue. After five days, corns and roots were truncated to exclude the corn as carbon source and to ensure highly branched roots. The next day, maize seedlings were planted into rhizoboxes with 135 g of fresh soil from the field. They were watered every day with 15 ml water. After one week 0.5 g l^{-1} KNO_3 was added to the water for five days. The labelling experiment was started 24 days after sprouting and the zero control plants were sampled. In both chambers 24 plants were inserted and $^{13}CO_2$ or $^{12}CO_2$, respectively were pumped into the chambers where a CO_2 concentration of 418 ± 27 ppm was established to ensure optimal carbon fixation rates. This concentration is only little more than the actual value of natural CO_2 concentration (about 390 ppm (Andrews *et al.* 2013)). Labelling lasted for 6 days but the experimental conditions were maintained for 16 days in total. Plants were watered during the experiment with 25-30 µl each day and 7.5 mg KNO_3 was added at day 4, 6, 9, and 12 as plants showed signs of nitrogen deficiency. Sampling took place after 0.5, 1, 2, 3, 5, 8, 12 and 16 days. For sampling, the fronts of the rhizoboxes were opened and soil was carefully removed with a spatula as the rhizoboxes were densely packed with roots. Then, Roots

were taken from the boxes and rhizosphere was sampled as described above. For each sampling point triplicate plants were sacrificed, but for RNA-SIP the soil was pooled again.

2.6.2. Detritussphere SIP labelling

The planning and practical realisation of this experiment was done by Susanne Kramer at University of Hohenheim. My contribution to this experiment was the downstream analysis of the substrate utilising bacteria and micro-eukaryotes by RNA-stable isotope probing, T-RFLP fingerprinting, amplicon pyrosequencing and evaluation of the resulting data sets for bacterial consumers. The data on micro-eukaryotes was analysed by Maike Hünninghaus (Department of Terrestrial Ecology, University of Cologne).

To set-up the microcosms, 50 g soil (dry weight) was filled into small metal cylinders. Each of four highly ^{13}C labelled (> 98 atom %) substrates (glucose, cellulose, maize leaves and maize roots) and respective ^{12}C control substrate were used. These substrates represented labile, more recalcitrant and complex substrates. Additionally, soil cylinders without any substrate were prepared as controls. Except of the ^{12}C glucose (Sigma Aldrich, St. Louis, USA) all substrates were delivered from IsoLife, Wageningen, Netherlands.

The carbon content of the cellulose and maize material was determined with an elemental-analyser vario MACRO (Elementar Analysensysteme GmbH, Hanau, Germany). For each microcosm, 12 mg (240 µg C g^{-1} soil) of substrate was added to the soil. To reach a good distribution of all substrates in the soil, maize plant material was milled (< 1 mm). Glucose was added in solution and cellulose and maize plant material as suspensions. The moisture content was adjusted to 60 % of the water holding capacity (whc) and the soil was mixed after adding the substrates for homogenous distribution. Then soil bulk density was set at 1.4 g cm^{-3} (average bulk density is 1.38 g cm^{-3} in topsoil of the field experiment). For respiration measurements, cylinders were placed in air-tight glasses which were closed at the top by a lid with a small vessel attached underneath and were filled with 1 M NaOH to absorb evolved CO_2. The microcosms were incubated in a climate chamber at 12 °C which is the long-time mean temperature of autumn months at the field site. ^{12}C treatments including controls were replicated three times; ^{13}C treatments were not replicated.

Water content was regularly checked by weighting the microcosms but no significant water content decrease could be observed. Microcosms were destructively sampled after 2, 8, 16 and 32 days.

At respective sampling days microcosm soil was mixed and samples for RNA-SIP were stored at -80 °C and for the other analyses at -24 °C. For NA-SIP analysis, soil from three ^{12}C replicate microcosms was mixed to obtain one representative ^{12}C sample.

2.6.3. Bacterial biomass SIP labelling

To identify inter-bacterial secondary trophic links, a microcosm experiment was planned with ^{13}C labelled bacteria. To ensure that this bacterial amendment was as realistic and natural as possible, I aimed to enrich root exudate utilisers directly from the soil. For this, several pre-experiments were done to develop the best enrichment method. Among the tested media, VL55 (Sait *et al.* 2002) achieved highest cell numbers and good diversity. As substrate, a mixture with known root exudate substrates was used (Marx *et al.* 2010). By testing several enrichment techniques with fluid and plate cultivation media I observed that transferring to fresh media always decreased the diversity of the enrichment culture. Therefore, I decided to use freshly grown enrichment cultures as inoculum without previous and time consuming analysis by T-RFLP to examine diversity or identity of the added bacteria. For enrichment, modified VL55 medium was prepared without the substrate:

1l VL55 contained

49.30 mg	$MgSO_4*7H_2O$
22.66 mg	$CaCl_2$
26.40 mg	$(NH_4)_2HPO_4$
1.95 g	MES (2-[N-morpholino]ethanesulfonic acid)
0.5 ml	selenite/tungstate solution (Tschech *et al.* 1984)
0.5 ml	SL10 (Widdel *et al.* 1983)
ad 997.5 ml	ddH_2O

pH was adapted to 5.5 and the medium was autoclaved at 120 °C for 20 min. After cooling to room temperature 2.5 ml of the vitamin solution (see below) were added.

selenite/tungstate solution:

0.5 g	NaOH
3 mg	Na_2SeO_3*5 H_20
4 mg	Na_2WO_4*2 H_2O
ad 1l	ddH_2O

SL10:

10 ml	25 % HCl
1.5 g	$FeCl_2$*4 H_2O
70 mg	$ZnCl_2$
100 mg	$MnCl_2$*4 H_2O
6 mg	H_3BO_3
190 mg	$CoCl_2$*6 H_2O
2 mg	$CuCl_2$*2 H_2O
24 mg	$NiCl_2$*6 H_2O
36 mg	Na_2MoO_4*2 H_2O
ad 1l	ddH_2O

vitamin solution:

2 mg	biotin
2 mg	folic acid
10 mg	pyridoxine-HCl
5 mg	thiamine-HCl*2 H_2O
5 mg	riboflavin
5 mg	nicotinic acid
5 mg	D-Ca-pantothenate
5 mg	vitamin B12
5 mg	p-aminobenzoic acid
5 mg	lipoic acid
ad 1l	ddH_2O

The Vitamin solution was filter sterilised after mixing.

Right before inoculation, 1 ml of artificial root exudate substrate solution was added to 100 ml of medium.

artificial root exudate substrate solution:

for 1l VL55 medium:

114.16 mg	glucose	≙ 1 mM l^{-1}
114.16 mg	fructose	≙ 1 mM l^{-1}
177.15 mg	sucrose	≙ 0.51 mM l^{-1}
91.97 mg	succinate	≙ 0.751 mM l^{-1}
62.08 mg	arginine	≙ 0.251 mM l^{-1}
54.05 mg	serine	≙ 0.51 mM l^{-1}
124.16 mg	cysteine	≙ 0.51 mM l^{-1}
27.67 mg	benzoate	≙ 0.211 mM l^{-1}
ad 10 ml	ddH_2O	

Substrate solutions were mixed both with ^{12}C and ^{13}C compounds and afterward filter sterilised.

For the enrichment culture, 1 g of fresh soil was added to 100 ml of ddH_2O and stirred for 15 minutes. 100 ml of medium were inoculated with 1 ml of a 1x 10^{-2} dilution of this soil extract and cells were counted with a Neubauer-improved counting chamber every two hours until cell density reached about 1x 10^8 cells ml^{-1}.

24 microcosms were prepared – 8 for each treatment (^{13}C bacterial inoculum, ^{12}C inoculum and no inoculum). 500 ml modified Schott flasks were used. Sieved soil was set to 60 % water holding capacity (whc) and left for four weeks for protozoa to adapt and germinate from spores. Every second or third day, flasks were opened for air exchange and the water holding capacity was adjusted if needed. Bacterial cell counts were estimated with 16S rRNA gene qPCR. At the day of inoculation with putative exudate utilising bacteria, enriched ^{12}C and ^{13}C bacterial cells were centrifuged (3345 g, 10 min, 4 °C), washed twice with base VL55 medium and resuspended such that after inoculation, 1x 10^8 cells g $soil^{-1}$ were added and water content was adjusted to 60 % whc. Suspensions with enriched bacteria were added in drops to the soil with constant stirring to ensure even distribution of enriched bacteria in the soil. Then, each microcosm was filled with 30 g of soil and the flasks were tapped on the table several times to compact the soil. Microcosms were sealed right afterwards. For sampling, whole microcosms were sacrificed and soil samples were frozen immediately for further analysis. Remaining microcosms were opened for 10 minutes to ensure good oxygen supply for the microorganism. As 90 % of the flask volume

was headspace, enough oxygen was present until the next sampling point. First sampling took place after half a day (10 hours), then after 1, 2, 3, 5, 8 and 12 days. RNA was extracted from all soil samples and from inoculum suspensions and analysed by stable isotope probing (see below) and subsequent T-RFLP fingerprinting for each fraction. To identify bacteria represented by distinct T-RFs, amplicon sequencing was performed for the fraction 2 with "heavy" RNA both for ^{12}C and ^{13}C treatments for samples taken after 1, 3, 8 and 12 days.

2.7. Stable isotope probing (SIP): ultracentrifugation gradients

To identify the bacteria and prokaryotes that incorporated carbon from labelled substrates in the specified experiments, RNA-stable isotope probing was used as described previously (Lueders 2010). Total RNA was quantified in three dilutions and each in triplicates with the Quant-iT™ RiboGreen® RNA Reagent and Kit (Life Technologies, Carlsbad, USA) according to the manufacturer's instructions but with a different standard curve (2.5, 2, 1.5, 1, 0.5, 0.1, 0.05 and 0 µg/ml). Fluorescence was measured with a Stratagene MX3000P qPCR cycler (Agilent, Santa Clara, Calif., USA).

For SIP gradients, 700 ng of ^{13}C- or ^{12}C RNA were mixed with 185 µl Formamide, ad 1ml gradient buffer (0.1 M Tris-HCl (pH 8), 0.1 M KCl, 1 mM EDTA, DEPC-water) and 5 ml CsTFA (buoyant density ~2g/ml, Amersham). Afterwards the refractory index was measured with a refractometer (AR200, Reichert technologies, Buffalo, USA) in temperature adjusting mode to assure a CsTFA density of about 1.80 g/ml. Otherwise the density was adjusted by addition of CsTFA or gradient buffer. The mix was transferred to polyallomer QuickSeal (BeckmanCoulter, Brea, USA) tubes which were than sealed and submitted to ultracentrifugation at 125.000 g for 65 h in a Centrikon T-2190 centrifuge (Kontron Instruments) with a VTI 65.2 vertical rotor (BeckmanCoulter, Brea, USA). No brakes were used for deceleration.

For fractionation of the gradient, the tube was gently fixed and a needle attached to a syringe pump filled with GIBCO® Water (Life Technologies, Carlsbad, USA) was inserted at the top of the tube. Then, the bottom of the tube was punctured and the pump was started at a speed of 1 ml per minute. Changing the collecting tube every 25 seconds, each gradient yielded 12 fraction of ca. 500 µl.

The refractory index was measured for each fraction and calculated into density based on a standard curve to be able to compare corresponding ^{12}C and ^{13}C fractions.

RNA was precipitated from the fractions with an equal volume of isopropanol and centrifugation at 20.000 rcf. The pellet was washed with 70 % ice cold ethanol, dried and then resolved in 25 µl EB buffer. Afterwards, the RNA of the fraction was analysed by RT-quantitative PCR, T-RFLP fingerprinting and, for selected fractions, by amplicon sequencing as described above.

2.7.1. Calculation of taxon-specific enrichment factors

To asses which identify bacteria clearly assimilated carbon from ^{13}C substrates, a pyrotag enrichment indicator was applied. This indicator is similar to the 'subtraction values' recently introduced for T-RFs (Zumsteg *et al.* 2013). However, here we consider read abundances from one light and one heavy fraction of both the respective ^{12}C and ^{13}C approaches.

Equation 2:

$$enrichment = \frac{\text{abundance 'heavy' } {}^{13}C}{\text{abundance 'light' } {}^{13}C} - \frac{\text{abundance 'heavy' } {}^{12}C}{\text{abundance 'light' } {}^{12}C}$$

We inferred enrichment factors for all taxa with a relative abundance >2 % (detritussphere experiments, biomass experiment) or > 1 % (rhizodeposit experiment) in heavy fractions in one of the ^{13}C treatments. All taxa with an enrichment factor >0.5 (detritussphere experiments, biomass experiment) or > 1 % (rhizodeposit experiment) where considered as ^{13}C-labelled. In the interpretation of our labelling results, not only these enrichment factors, but also absolute rRNA read abundance of a given taxa in 'heavy' rRNA, as well as labelling patterns evident from T-RF abundance across the entire SIP gradients were considered.

2.7.2. Calculation of cell numbers

Cell numbers of specific bacterial taxa were estimated from pyrosequencing and qPCR data together with taxon specific 16S rRNA gene copy numbers per cell derived from the IMG data base (Markowitz *et al.* 2012). The calculation is depicted in Formula 2. Cell numbers were calculated from pyrotag relative read abundances of identified key taxa which were multiplied with absolute 16S RNA gene counts from qPCR analysis of the same soil sample. This gave an approximation of taxon-specific 16S RNA gene counts per g soil for these bacteria. Subsequently, to correct for biases from varying 16S rRNA

operon copy numbers in distinct genomes, total copy numbers were divided by taxon specific operon copy numbers per cell. By this, specific cell abundance was estimated for each taxon in the field:

Equation 3:

$$\text{specific cell number} = \frac{\text{specific relative sequence abundance} \times \text{total copy number (g soil)}^{-1}}{\text{specific avarage copy numbers cell}^{-1}}$$

2.8. Inference of bacterial biomass carbon

Two estimation approaches were used to infer bacterial biomass carbon in lysimeter samples by the means of the qPCR data. (A) In the main approach, we determined pyrotag read abundances for the most important phylogenetic lineages representing over 90% of all sequence reads in each sample. Then, the average rrn operon copy number per cell was searched at IMG (Markowitz et al., 2012) for these families and lineages. Subsequently, an extensive literature search was done for a reasonable estimate of the average carbon content per bacterial cell in agricultural soils. Unfortunately, almost all respective studies refer to marine bacteria and pure or enrichment cultures and we found a wide range of 1.17 - 214 fg carbon per cell (Bratbak, 1985; Fagerbakke et al., 1996; Fukuda et al., 1998; Loferer-Krossbacher et al., 1998; Simon and Farooq, 1989; Tuomi et al., 1995; Watson et al., 1977). We decided to adapt values from Trousellier et al. (1997), who used cultures of five marine and five non-marine species under starving conditions and reported an average of 26.42 ± 1.08 fg carbon per cell, which is in an adequate range. (B) As control, we consulted the data on microbial biomass carbon obtained by chloroform-fumigation from the same soil samples (Kramer et al., 2012) and correlated those with 16S rRNA gene qPCR counts from the same samples and depths. However, since only data for total microbial biomass carbon was available (including prokaryotes and microeukaryotes), we estimated the fungi: bacteria biomass ratio by following Joergensen and Wichern (2008) who reviewed a bacterial contribution of 40 % - 85 % to total microbial carbon in agricultural soils.

2.9. Statistics

All data used for statistics were standardised first with the arcsin(√x) transformation which is recommended for percentages (Ramette 2007) and then normalised with the chord transformation to give less weight to rare species, to reduce bias from zero values and therefore, to be able to use linear statistical methods (Legendre *et al.* 2001).

All statistical analysis were done with the open source statistical software environment R (R Development Core Team 2011).

2.9.1. Principal component analysis (PCA)

First, unbiased evaluation of the tempo-spatial abundances of the bacteria in the field was accomplished by PCA of the T-RFLP fingerprinting data to detect distribution patterns without presumptions. For principal component analysis (PCA) the rda function of the vegan package (Oksanen *et al.* 2012) was used in a unconstrained manner: with a variance-covariance matrix only of the dependent variables and without explanatory variables as recommended previously for PCA of environmental data sets (Borcard *et al.* 2011b). Both sites and species were scaled symmetrically by square root of eigenvalues. PCA was than depicted as biplot.

2.9.2. Multivariate analysis of variance (MANOVA)

Impact and significance of explanatory variables on the variance of the bacterial community composition were assessed by multivariate analysis of variance (MANOVA) with the T-RFLP fingerprinting data. This was done with the program adonis from the R package vegan (Oksanen *et al.* 2012) with a Bray-Curtis distance matrix and 200 permutations.

2.9.3. Multivariate regression tree (MRT)

To analyse and weight factors influencing the bacterial distribution in the field, multivariate regression tree analysis (MRT) was performed with the function mvpart from the mvpart package (De'ath *et al.* 2012). MRT is a complex but robust and powerful model to evaluate ecological data sets. It divides the environmental variables into increasingly smaller groups with the end that the within-group deviances are minimised and homogeneities are maximised. Splitting is done with respect to the explanatory variables and therefore, variable characteristics can be identified for each group. (Borcard *et al.* 2011a). Splitting criteria and measure of homogeneity was within-group sums of squares about the group means (Euclidian distance). Tree size was selected such, that the cross-validated relative error was at minimum plus one standard error, as it is recommended (De'ath *et al.* 2000). The result of the MRT clustering was visualized in a tree structure as it is usually done but also as PCA biplot to identify the species that are important for

certain groups. This could be done as both MRT and PCA used the same metric (De'ath *et al.* 2000).

2.9.4. Redundancy analysis (RDA)

The impact of measured soil properties on the variance of the bacterial soil community caused by the determined environmental variables was analysed by RDA. As information on total organic carbon (TOC), extractable organic carbon (EOC), total nitrogen (TN) and water content were only ascertained for bulk soil samples, only those T-RFLP fingerprints were considered here. RDA is a constrained ordination analysis, where only the variation of the community composition that can be explained by the environmental variables is represented. RDA was depicted as triplot with samples, T-RFLPs and environmental (dependent and independent) variables. With this, impact and coherence of environmental variables can be revealed.

2.9.5. Functional organisation analysis (*Fo*)

It is not recommended to derive the Shannon-Wiener diversity index *H'* from T-RFLP data, as the numbers of rare taxonomic units can be considerable (Blackwood *et al.* 2007). Therefore, the *Fo* index was applied here for the T-RFs of 'heavy' SIP gradient fractions to assess the diversity of experimentally identified bacterial substrate consumers. *Fo* is a method based on the Pareto-Lorenz evenness curve (Marzorati *et al.* 2008), and as in *H'*, community richness and relative abundances of individual taxa are implemented in *Fo*. However, rare taxa are less important as cumulative relative OTU abundances are ranked on the X-axis of the Pareto-Lorenz curve and the cumulative relative OTU proportion of 20 % of all taxa is derived. This *Fo* index would be 0.2 at perfect evenness. A relatively high *Fo* index means a bacterial community of low diversity and with few dominant taxa.

3. Results

This thesis project was part of the DFG Research Unit FOR 918 ('Carbon flow in belowground food webs assessed by stable isotope tracers'). The Research Unit aims to investigate carbon flow through a soil food web and its key organisms of all trophic levels, as well as the links and carbon fluxes between them, in an elaborate and integrated approach. An agricultural field was set up and sampled in May, July, September, December and following July to cover all seasons and plant growth stages. Furthermore, we took the samples from three depths: top soil (0-10 cm), below plough layer (40-50 cm) and from subsoil (60-70 cm). Maize (a C_4-plant) was grown on a field that was previously cultivated with only C_3-plants over decades, and food web members from meso- and macrofauna were traced by carbon isotope signatures. To detect effects of substrate quality on the food web, replicate field plots were cultivated with fodder maize (whole shoot harvested), corn maize (shoot litter added to soil after harvest), wheat with maize litter and wheat as controls.

As bacteria still are treated as a 'black box' in soil food web, where carbon fluxes just enter and exit a unspecified biomass, this thesis project aimed to specifically unravel bacterial food web members. Identification was realised by microcosm experiments and stable isotope probing (SIP), and then the distribution and abundance of respective key players was quantified in the field. Moreover, field bacteria mobilised by seepage water were investigated and their contribution to transported carbon into the subsoil.

3.1. Field sampling and investigation of the bacterial community distribution *in situ*

One aim of the field sampling campaigns was to evaluate the abundances and distributions of to-be-identified bacterial food web members with regards to soil compartment (bulk soil, rhizosphere and root surface), soil depth, sampling time and cultivation treatment. Prior, the overall composition of the field bacterial community was analysed, and the effects of determined variables on the bacterial distribution in the field were evaluated.

3.1.1. Spatial homogeneity of the bacterial community in the field

First, spatial homogeneity of the field bacteria over transects of length and depth was assessed, with a preliminary sampling conducted before actual experimental setup. Soil samples were taken in triplicates from top soil with distances of 0, 0.2, 1, 5, 25 and 125 m along a straight line from NE to SW. For the depth transect, samples were taken at 5 cm,

35 cm and 65 cm depth. Heterogeneity of the bacterial community in these samples was than analysed by replicate bacterial 16s rRNA gene T-RFLP fingerprinting.

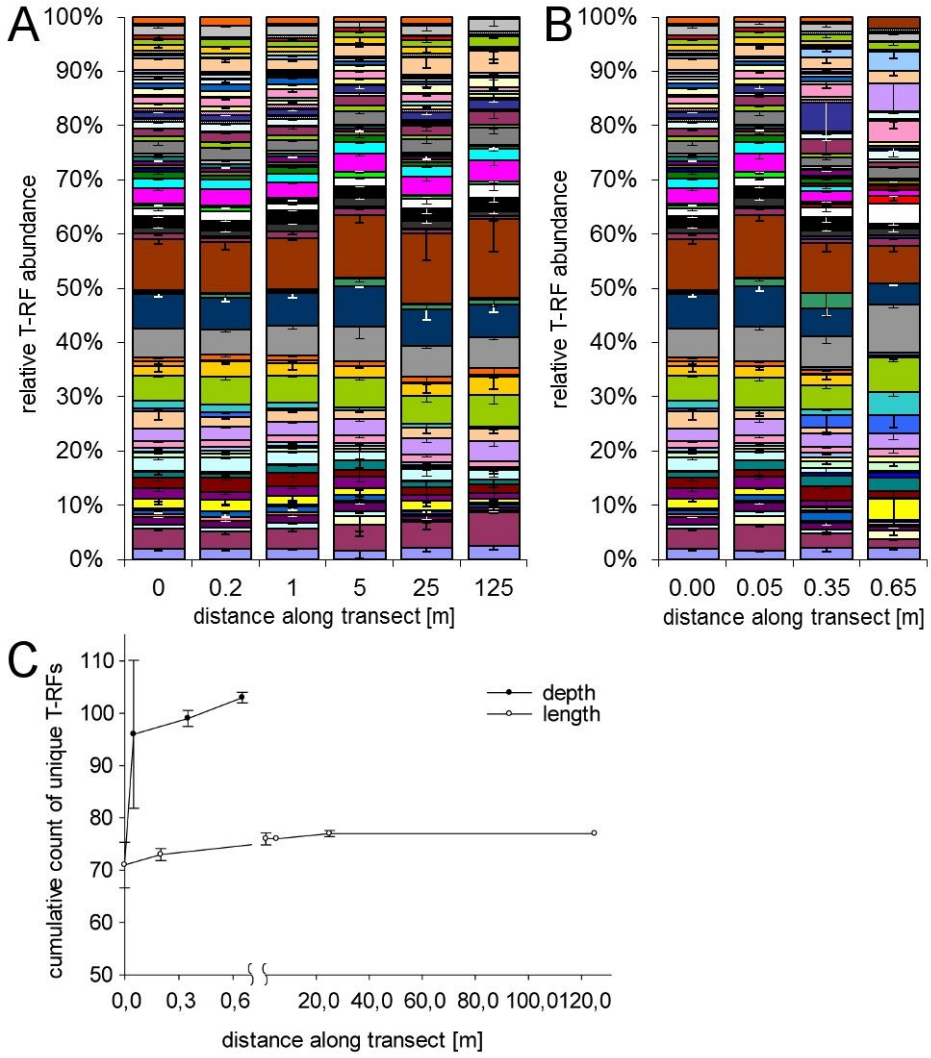

Fig. 9: Horizontal and vertical homogeneity of the bacterial community in the field in March 2009. Bar plots with relative T-RF abundances for the horizontal transect sampled from top soil (A) and from the vertical transect (B) with standard deviations of three replicates. C: Increase of cumulative unique T-RFs detected over the transects

The overview of T-RF abundances in Fig. 9 revealed a quite diverse bacterial community in each sample with T-RFs of varying abundances and no pronounced OTU dominances. The T-RF distribution pattern was very similar for all samples of the length transect (Fig. 9A), but appeared to change with depth (Fig. 9B). This impression was confirmed by analysis of the amount of cumulative overall T-RFs occurring over the horizontal transect line. Beyond a distance of only one meter (replicate samples), hardly any new T-RFs were observed for the horizontal transect in contrast to the vertical transect (Fig. 9C).

Generally, T-RFLP fingerprinting is a very robust and reproducible method for bacterial community analysis (Osborn *et al.* 2000). In the data set of the actual field sampling 75 % of the overall T-RFs were present in all triplicates and 97 % of the more abundant T-RFs (> 1 %). Mean relative standard deviation of T-RFs present in all triplicates was 30 % (min.: 0 %, max.: 150 %).

Results from the length and depth transect were analysed together by principal component analysis (PCA) and it became obvious that the composition of the bacterial soil community changed vertically much more over 0.65 m of depth than horizontally over 125 m (Fig. 10). Especially, as the first principal component - mostly determined by depth - explained much more of the overall variance than the second, which was mostly affected by horizontal distance.

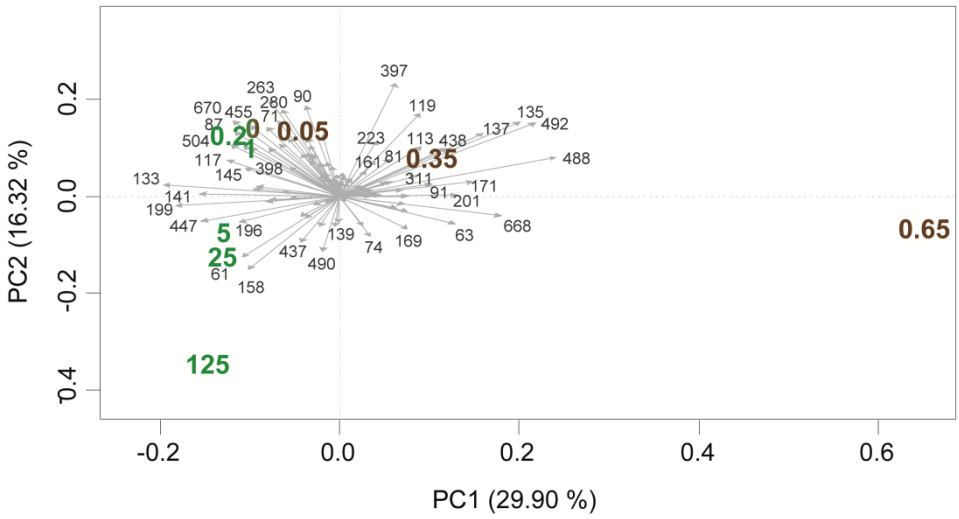

Fig. 10: PCA biplot of T-RFLP fingerprinting results of both the horizontal (green bold letters) and vertical transect (brown bold letters). T-RFs are depicted as arrows (direction = group allocation; length = impact) and only the relevant T-RFs are denoted. Distances of samples are denoted in metres.

3.1.2. Spatio-temporal dynamics of the bacterial community during the field experiment

For the field experiment, soil samples were taken in May, July, September and December 2009 to cover all seasons and plant growth stages. Additionally, samples were taken in the subsequent July to assess the stability of seasonal influences. Three depths were sampled regarding horizon structures of the exploratory field: 0 -10 cm (top soil), 40 - 50 cm (below plough sole) and 60 – 70 cm (subsoil). For each treatment, respective depth sections of 10 soil cores were pooled to minimize effects of spatial heterogeneity vs. treatments on sampled microbiota. Composite samples were taken as biological triplicates from three plots with the same cultivation treatment. Rhizosphere and root surface were only sampled for corn maize. Plot segmentation and cultivation are depicted in

Fig. 8.

The bacterial community was first investigated by T-RFLP fingerprinting with 16S rRNA gene targeted primers. All in all, 148 samples were analysed for this data set, making it too complex to evaluate differences and influence of environmental variables by visual inspection of raw data. Therefore, sophisticated multivariate statistic tools were applied which were mostly selected due to robustness and appropriate statistical requirements for the data. All data were normalised by chord transformation and standardised prior to statistical analysis to minimise methodological errors (Ramette 2007). First, to get an unbiased overview without constricting presumptions, principal component analysis (PCA) was used for samples (Fig. 11). Here, above all, root surface and rhizosphere samples were separated from the bulk soil samples in the direction of the first principal component (PC1). Several T-RFs were much more abundant at root surface and in rhizosphere samples in comparison to bulk soil. Especially, T-RFs of 495 bp, 131 bp and 435 bp were specific for maize root surface samples. Characteristic T-RFs for rhizosphere samples were mostly 87 bp, 263 bp, 124 bp and 149 bp. Bulk soil samples were mostly divided by depth along the second principal component (PC2), where samples from top soil and root free zone formed distinct clusters. The samples from the middle layer at the plough sole spread in between but also into the clusters of the other two depths. Samples from the deep root free zone were mainly characterised by the T-RFs of 668 bp, 146 bp and 490 bp. Among others, T-RFs of 133 bp, 117 bp, 290 bp and 540 bp were typical for top soil samples (Fig. 11).

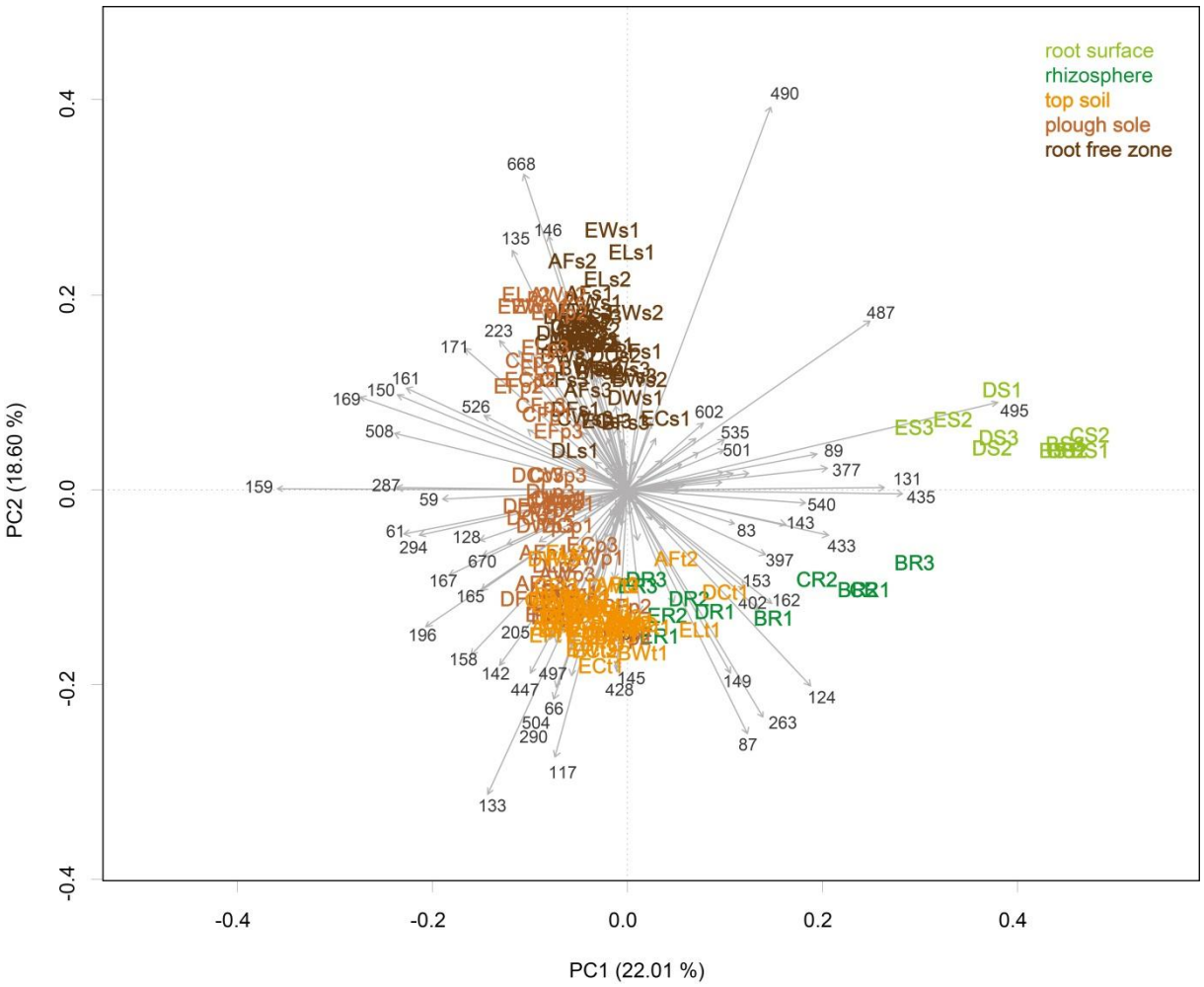

Fig. 11: PCA biplot of 16S rRNA gene T-RFLP fingerprints from all analysed soil samples. The colours designate the spatial origin of the samples, other variables are encoded in the sample names. first letter = time point: A = May '09, B = June '09, C = September '09, D = December '09, E = June '10. second letter = treatment: W = wheat, L = wheat + maize litter, F = fodder maize, C = corn maize. t = top soil, p = plough sole, s = subsoil. 1, 2 and 3 denote replicate numbers. T-RFs (in bp) are depicted as arrows (direction = sample allocation; length = impact) and only the more relevant are denoted.

Clustering due to cultivation treatment and time point was not apparent by PCA within the total data set (Fig. 10). To evaluate the influence of all determined variables, ADONIS (Anderson 2001) was performed. ADONIS is a multivariate analysis of variance (MANOVA) based on dissimilarity matrices instead of Euclidian distances and the outcome is listed in Table 2. In fact, all variables were causing significant influence ($p < 0.005$) on the variance of the samples except of field plot, resembling variance among triplicates. This again demonstrates the horizontal homogeneity of the bacterial community in the field. As already apparent from the PCA (Fig. 11), the most important discriminators were depth and soil compartment (bulk soil, rhizosphere or root surface). Time point of

sampling and cultivation treatment influenced the bacterial community of the exploratory field as well, but only to minor extent (Table 2).

Table 2: Correlation coefficients (R^2) and p-values of the determined independent environmental variables as determined by ADONIS multivariate analysis of variance.

	R^2	p
depth	0.22	<0.005
soil compartment	0.24	<0.005
treatment	0.06	<0.005
time	0.09	<0.005
plot	0.02	0.07

To evaluate, how samples differed and grouped in relation to the environmental variables, a multivariate regression tree was used. Tree size was determined by the minimum of the cross validated error plus one standard deviation, as recommended (De'ath *et al.* 2000). Grouping criterion was minimised in-group sum of squares about the mean. As already obvious from PCA and MANOVA, soil compartment and depth were the most important discriminators and root surface was separated from all other samples first (Fig. 12). Then, samples from the subsoil were separated from higher bulk soil and rhizosphere samples. In the next step, top soil and rhizosphere samples were divided from those of the plough sole layer and only then rhizosphere was separated from top bulk soil. Further, root surface and rhizosphere samples from corn maize plots were split into an early group for July and September 2009 and a late group for December 2009 and July 2010. Bacterial communities in the bulk soil samples were more dependent on seasonal changes and both samples from top soil and root free zone were divided into a group for Julys (both years) and a group for all other time points (Fig. 12A). In Fig. 12B, these different groups are placed in a PCA biplot, which is very much identical to the ordination observed in unbiased PCA (Fig. 11). However, T-RFs were assigned more specifically to given sample groups here than with the previous PCA.

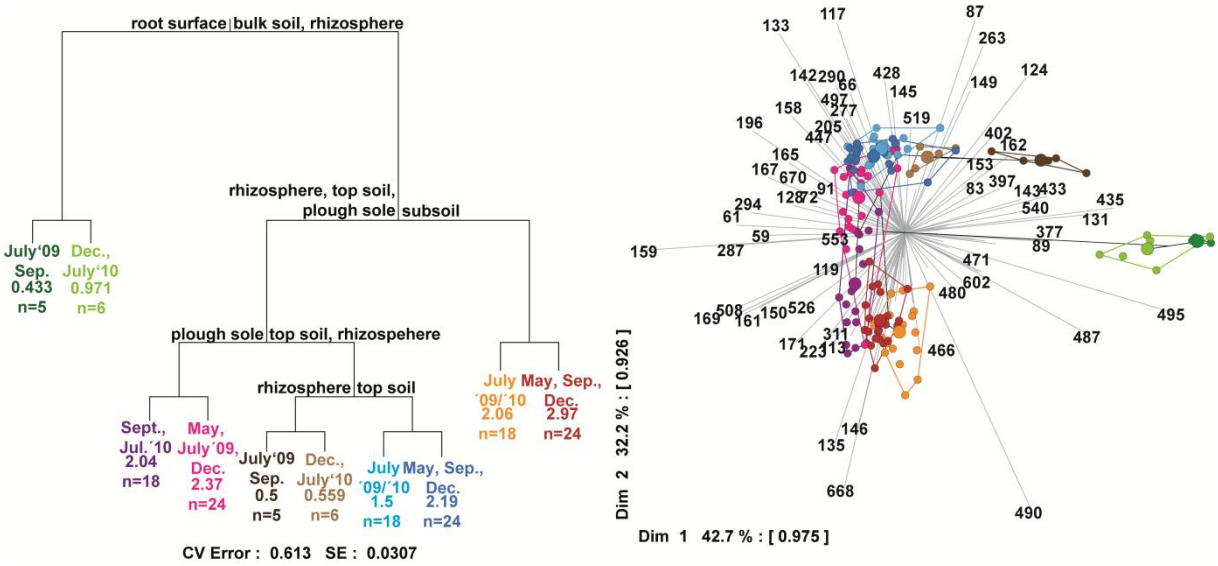

Fig. 12: Tree (A) and PCA biplot (B) from the Multiple Regression Tree. (A): Values below the group designation are the group sum of the squares about the group mean; n is the number of samples in the group. CV error is cross-validated relative error and SE standard error. (B): Groups defined by MRT are in the same colour as in (A). Small circles represent samples, big ones group means. Influences of T-RFs (in bp) to the grouping are depicted by direction (group allocation) and length (impact) of the grey vectors. Only important T-RFs are labelled.

For early maize rhizosphere, T-RFs of 143 bp, 433 bp and 162 bp were specific, and in late maize rhizosphere T-RFs of 149 bp, 263 bp and 87 bp were more abundant. The subsoil samples were mainly characterised by T-RFs of 490 bp and 466 bp in July, and for all other time points by T-RFs of 668 bp, 135 bp and 146 bp. The subgroup of the plough sole samples, however, overlapped strongly with those from top soil and subsoil, and assignment of T-RFs characteristic for the particular groups was not feasible. For all group splits because of seasonal no specific T-RFs could be defined.

To understand, what soil characteristics chiefly influenced the differences in the bulk soil bacterial community composition due to depth, season and cultivation treatment, a redundancy analysis (RDA) was performed with determined independent variables and measured soil properties as dependent variables. Soil water content, total and extractable organic carbon (TOC, EOC) were measured by Susanne Kramer from University of Hohenheim (Kramer *et al.* 2013) and total nitrogen (TN) by Nicole Scheunemann from the University of Göttingen (Scheunemann *et al.* 2010) for the samples from both Julys, September and December. Mean top soil TOC content was 1.12 % ± 0.03 and for subsoil 0.33 % ± 0.19. TN content was on average 0.11 % ± 0.002 for top soil and 0.04 % ± 0.02 for subsoil. The triplot in Fig. 13 confirmed again that depth had great impact on bacterial community composition. TOC, EOC as well as TN were important soil properties

declining with depth, as their vectors had similar planes but opposed directions as depth in the RDA plot.

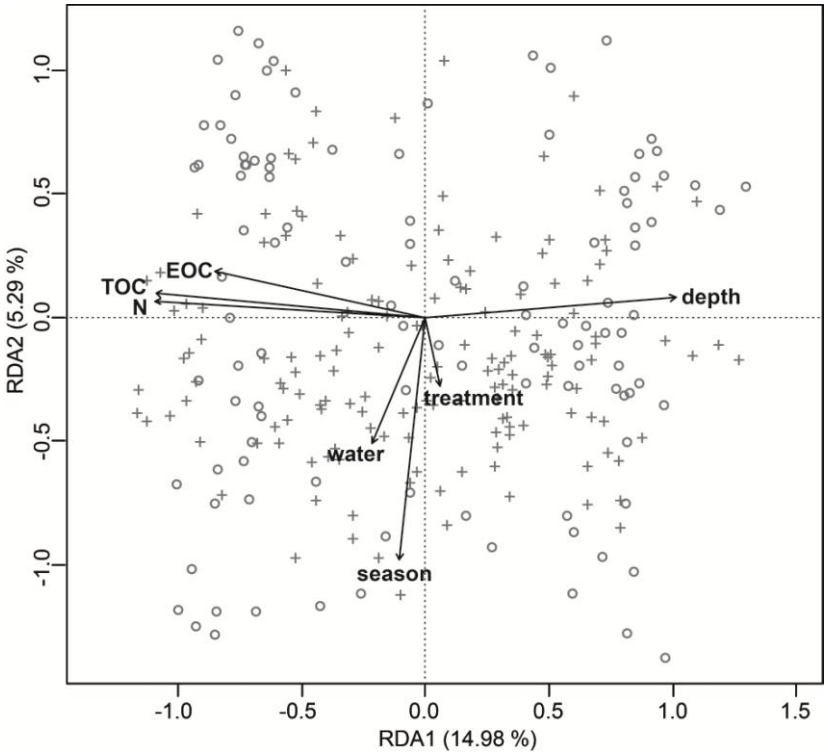

Fig. 13: RDA triplot of fingerprinting data from the field site. Vectors show the influence of dependent and independent environmental variables on the bacterial community composition. Samples (○) and T-RFs (+) are depicted as symbols.

With increasing depth, TOC and TN decreased. Water content, however, correlated rather with seasonal changes. Other important parameters like pH were only measured once in the course of the initial pedological evaluation of the field and were therefore not included in the RDA. But as pH (H_2O) increased with depth from 6.6 to 7.7 and pH ($CaCl_2$) from 6.0 to 7.0 (Kramer *et al.* 2012), it can be assumed that pH had also a strong influence on the variation of the bacterial community associated with depth.

Amount of bacteria in the field soil was evaluated by 16S rRNA gene-targeted qPCR (Fig. 14). Gene abundances were always highest in rhizosphere samples and for bulk soil, copy numbers degreased with depth for all sampling time points. Highest counts were obtained in top soil and rhizosphere in summer. In contrast, 2-4 times more 16S rRNA gene copies were observed for root surface samples in winter compared to summer samples.

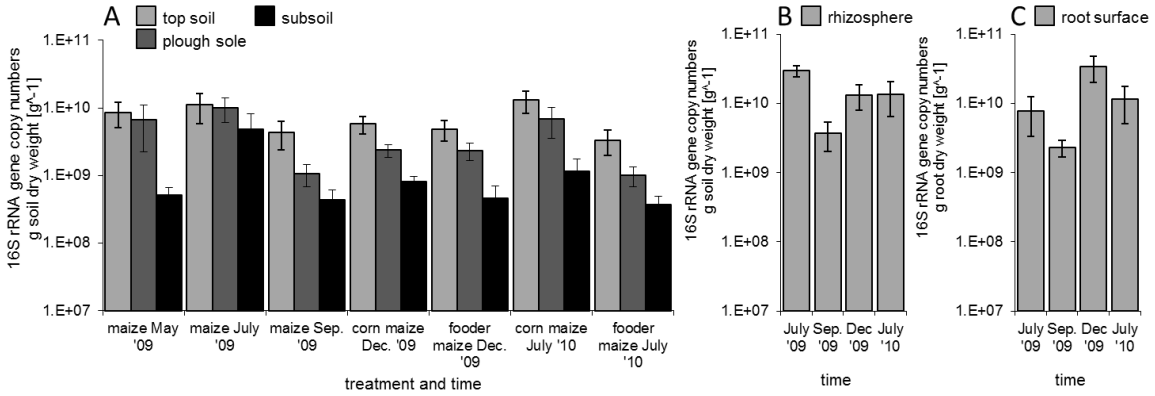

Fig. 14: Copy numbers of 16S rRNA genes in the bulk soil (A) and rhizosphere (B) and at root surfaces (C). Counts from bulk soil and rhizosphere samples were set in relation to g soil (dry weight) and counts from root surface relate to g root (dry weight).

3.1.3. Comparison of RNA and DNA fingerprints

To assess distinctions between active bacteria and the overall soil community, 16S rRNA T-RFLP fingerprints were generated in triplicates for one representative wheat and one corn maize plot in triplicates. Corresponding fingerprints on RNA and DNA level were statistically analysed by PCA (Fig.15). Most obviously, root surface fingerprints again grouped together, isolated from bulk soil and rhizosphere samples, regardless of the examined nucleic acids. Particularly, T-RFs of 131 bp, but also 377 bp, 89 bp, and 435 bp were characteristic for this grouping which was in line with the PCA for all DNA fingerprints (Fig. 11, Fig. 12b). For bulk soil and rhizosphere samples, however, fingerprints at RNA and DNA level formed distinct groups. Especially, bacteria with a T-RF of 149 bp and 61 bp and also, for example, 145 bp, 469 bp and 153 bp were especially abundant in soil RNA. In contrast, T-RFs of 80 bp, 490 bp and 159 bp were much more abundant for DNA than for RNA fingerprints, which could indicate dormant or even dead cells.

Both RNA and DNA soil fingerprints formed a gradient in the PCA biplot from top soil to deeper soil samples. This depth gradient had the same orientation as the separation of RNA and DNA fingerprints with the top soil samples rather spread towards RNA ordination. This may indicate a generally higher activity of bacteria in top soil samples compared to deeper soil layers. Rhizosphere rRNA samples grouped near top bulk soil samples, but even closer to the root surface samples. Rhizosphere DNA samples, however, were spread between root surface to top soil samples. No seasonal influence on the bacterial community composition was detectable in PCA analysis here.

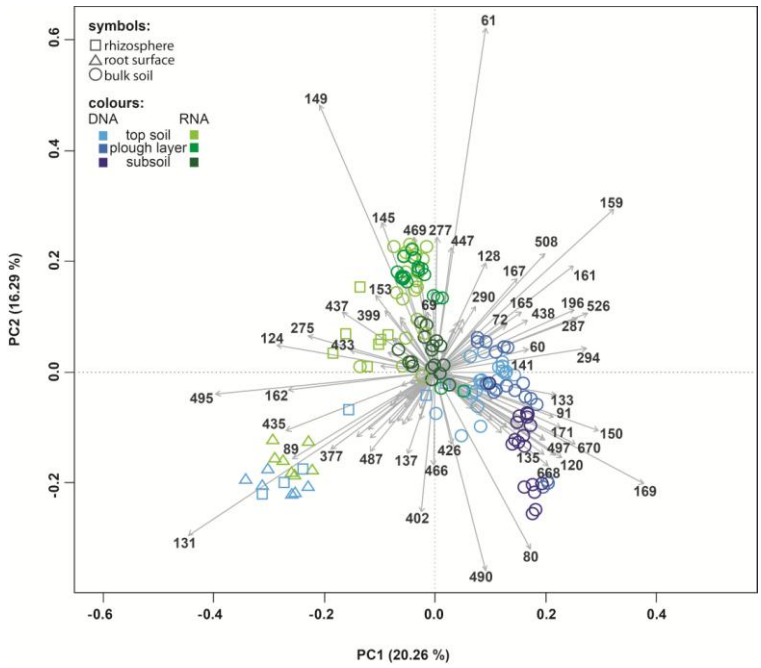

Fig.15: PCA biplot of rRNA and DNA fingerprints from all four sampling points in 2009. rRNA fingerprints are coloured in green, DNA fingerprints in blue. Colour shades indicate soil depth with top soil samples shaded lightest, subsoil samples shaded dark. Circles depict bulks soil, squares rhizosphere and triangles root surface samples.

In summary, the bacterial community was very homogeneous over the horizontal expansion of the field, but significant variation was observed for depth, soil compartment (root surface, rhizosphere and bulk soil), and also (to minor extent) for sampling time and cultivation treatment. Highest 16S rRNA gene copy abundance was observed in rhizosphere and top soils, with a steep decline over depth. rRNA fingerprints hinted towards a generally higher bacterial activity at the root surface, in the rhizosphere and for top soil samples.

3.2. Identification of key bacterial food web members by stable isotope probing

To identify bacteria actively taking part in the soil food web, three microcosm experiments were conducted within the Research Unit. For each experiment different ^{13}C-labelled substrates were used, representing the varying composition and stability of relevant natural substrates. Rhizodeposit users were labelled by exposing potted maize plants to $^{13}CO_2$, which then released ^{13}C-rhizodeposits into the soil via their roots. Microorganisms degrading plant litter and related compounds were labelled in microcosms with ^{13}C-glucose, -cellulose, -maize leaf and -maize root litter. To reveal intra-bacterial trophic interactions, putative rhizodeposit-utilising bacteria themselves were labelled with artificial

^{13}C-labelled model root exudates, and then added to soil microcosms. Experiments were planned and conducted by different members of the Research Unit and myself. I analysed the substrate utilising bacteria from these experiments. Fungi as well as protists were analysed from the same microcosm samples by the respective partners (fungi: Julia Moll, group F. Buscot, UFZ Halle/Leipzig; protozoa: Maike Hünninghaus, group M. Bonkowski, University of Köln). By this, we identified not only primary substrate utilisers, but also revealed food web links between microbiota. Besides the conduction of the microcosm experiment with labelled biomass, my own specific contribution to the labelling experiments was involvement in planning and conducting the experiments, as well as the actual conduction and data evaluation of all SIP gradients, as well as bacterial T-RFLP fingerprinting and amplicon sequencing analyses for all three experiments.

3.2.1. Rhizodeposit SIP experiment

In a microcosm experiment with maize plants under $^{13}CO_2$ atmosphere, rhizodeposit-utilising bacteria were identified by SIP. The experiment was planned and conducted by Maike Hünninghaus and Robert Koller (University of Cologne) with my help. Further, I identified the labelled bacterial food web members. The experiment lasted for 16 days and labelling was done throughout the first 6 days under a slightly elevated partial pressure of $^{13}CO_2$ (418 ± 27 ppm). Temperature and light conditions mimicked natural summer weather in the field. Rhizobox soil samples from day 1, 3, 5, 8, 11 and 16 and rhizosphere samples from day 5 and 8 were analysed with RNA-SIP and subsequent T-RFLP fingerprinting of the RNA gradient fractions. (Bar plots of all relative T-RFLP fingerprints from the distinct fractions are in appendix Fig. A.1). Labelled T-RFs were identified by comparison of T-RF abundances of 'heavy' with 'light' rRNA fractions from the ^{13}C treatments, and by comparison with 'heavy' fractions of ^{12}C-controls. Only if a T-RF was noticeably more abundant in 'heavy' ^{13}C fractions than in all other ^{12}C and ^{13}C fractions, it was considered as labelled.

For days 1, 3 and 16 no labelled T-RFs were observed. On the other days, only few labelled T-RFs were identified (Table 3) in the rhizobox soil. At day 5, T-RFs of 428 bp, 447 bp and 487 bp were labelled, the T-RFs of 132 bp, 447 bp and 487 bp on day 8, and the T-RFs of 447 bp and 487 bp on day 11. Because of this initially low number of labelled T-RFs in the overall rhizobox soil, rhizosphere soil was also sampled by root shaking and washing, although the soil of the rhizoboxes was already densely packed with roots. However, also for these distinct rhizosphere samples, only few T-RFs were labelled on day

5 and 8, similar as in mixed rhizobox soil samples. On day 5, one T-RF with 71 bp appeared weakly labelled. On day 8, T-RFs with 147 bp, 435 bp and 447 bp were ^{13}C enriched in the rhizosphere (Table 3).

Table 3: Labelled T-RFs in the rhizosphere SIP experiment with assigned taxa. exp. T-RF: T-RF recognized as labelled by comparing T-RFLP fingerprint of SIP gradient fractions from ^{13}C treatments and ^{12}C-controls; in-silico T-RFs: best matching T-RF found in according contig sequences. n.d.: no distinct taxa were detected for this T-RF.

	Day 5			Day 8		
	exp. T-RF	taxa	*in-silico* T-RFs	exp. T-RF	taxa	*in-silico* T-RFs
rhizobox soil	428	n.d.		132	n.d.	
	447	*Opitutus*	451	447	*Opitutus*	450
	487	*Opitutus*	487	487	*Opitutus*	487
rhizosphere	71	*Arthrobacter*	67	147	*Azospirillum*	150
				435	*Mucilaginibacter*	435
				447	*Opitutus*	450

As most labelled T-RFs were found on days 5 and 8, fractions 3 (‘heavy’) and 8 (‘light’) from ^{13}C and ^{12}C gradients were sequenced by pyrotag analyses to identify the bacteria represented by these T-RFs. Sequence reads were assembled into contigs containing both forward and reverse primers, and *in-silico* T-RF lengths were identified. Thus, labelled genera could be correlated to labelled T-RFs from the fingerprinting analysis (Table 3). Furthermore, additional putative rhizodeposit users were determined by enrichment indicators which were calculated for each taxon. This is an approach analogous to the ‘T-RF subtraction values’ introduced recently for SIP (Zumsteg *et al.* 2013), but in my approach, ‘heavy’ and ‘light’ fractions from both ^{12}C and ^{13}C treatment are regarded.

Taxa with an enrichment > 1 and relative sequence abundance more than 1 % in the “heavy” ^{13}C fraction were determined as labelled (Fig. 16).

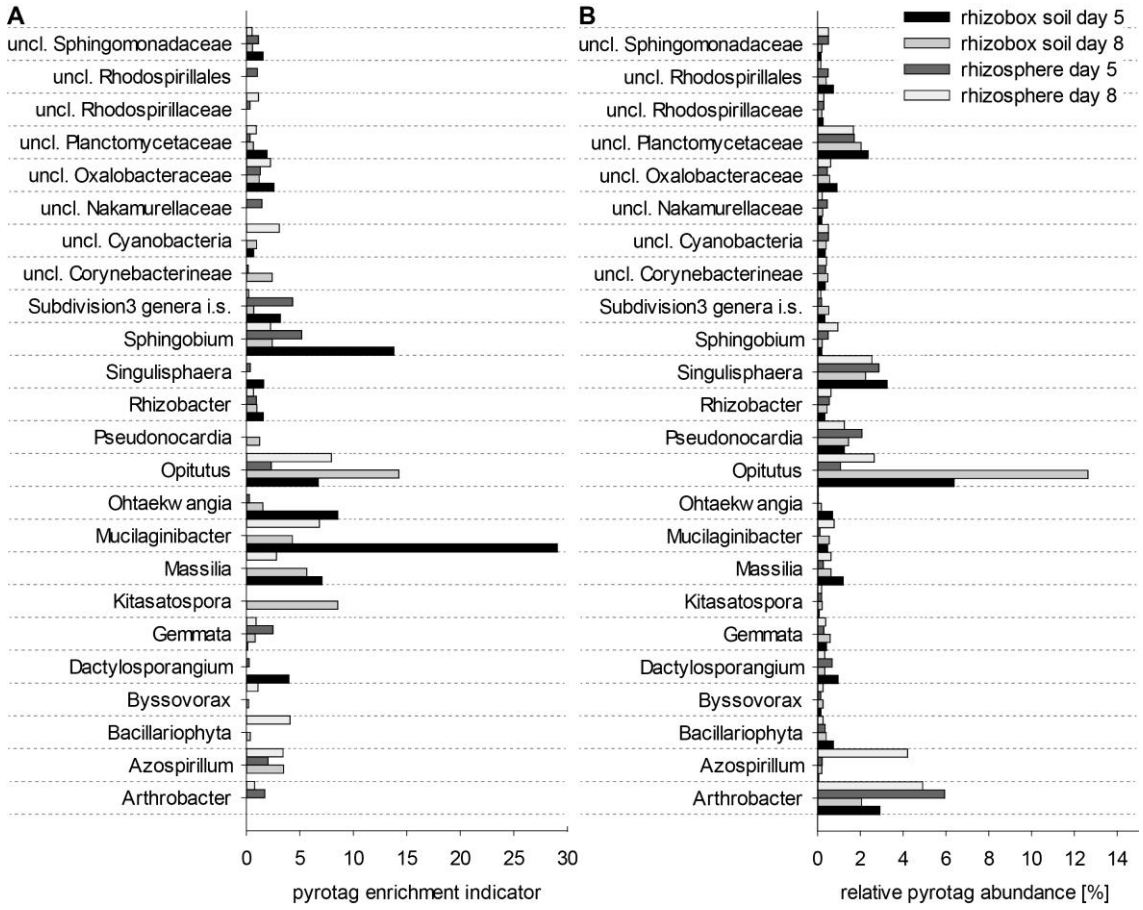

Fig. 16: Enrichment indicators (A) and relative sequence abundance (B) in "heavy" ^{13}C-rRNA fractions for bacterial taxa labelled by ^{13}C-rhizodeposits in mixed soil and in rhizosphere on day 5 and 8 of the experiment. Taxa with an enrichment > 1 and > 1 % relative sequence abundance were considered as labelled.

Although some of the labelled taxa were highly enriched, *e.g. Mucilaginibacter* spp. at day 5, most of the labelled genera were not very frequent in the soil rRNA and their relative sequence abundances were low. Therefore, it is likely that not all labelled taxa could be detected by T-RFLP fingerprinting. Furthermore, it is well-known that one T-RF can comprise amplicons of several microbial taxa which would mask the labelling as well.

Besides *Mucilaginibacter* spp. (*Bacteroidetes*), also *Sphingobium* spp. (*Alphaproteobacteria*), *Massilia* spp. (*Betaproteobacteria*), *Ohtaekwangia* spp. (*Bacteroidetes*) and *Opitutus* spp. (*Verrucomicrobia*) were clearly labelled after 5 days. At day 8, two days after labelling stopped, fewer genera were distinctly labelled (*Opitutus* spp., *Massilia* spp., *Kitasatospora* spp. (*Actinobacteria*)). Some of the rhizosphere utilising bacteria were abundant in the 'heavy' fractions but less distinct labelled (*e.g. Arthrobacter* spp. (*Actinobacteria*), *Azospirillum* spp.(*Alphaproteobacteria*) *Singulisphaera* spp.

(*Planctomycetes*)). For rhizosphere samples, similar taxa were labelled however, less enriched (Fig. 16).

Generally, rhizodeposits were used of a diverse and temporally variable bacterial population with no clearly dominating taxa. Although only a small number of bacterial 16S rRNA OTUs was found to be enriched in ^{13}C by T-RFLP fingerprinting, additional taxa with low-abundances genera were found to be by pyrotag sequencing. This discrepancy results from the generally low abundance of ^{13}C enriched taxa in our experiment. Hence, T-RFs of most labelled bacteria were probably masked by unlabelled rRNA with the same T-RFs, and therefore not detected in the fingerprint approach. Only the more abundant labelled taxa, i.e. *Opitutus* spp., were clearly recognized as ^{13}C enriched via both T-RFLP fingerprinting and pyrotag sequencing.

3.2.2. Detritussphere SIP experiment

Litter degraders and subsequent trophic links were to be identified in this microcosm experiment, conducted at the University of Hohenheim by Susanne Kramer, with assistance in planning from my side. Furthermore, I analysed bacterial RNA from the soil with SIP gradients, T-RFLP fingerprinting. Amplicon sequencing to identify bacterial degraders of the added substrates was also done by me, assisted by Susanne Kramer. Microcosms with either ^{13}C-glucose, cellulose, leaf litter or root litter were established to address the varying complexity and recalcitrance of natural detritussphere substrates. Sampling occurred after 2, 8, 16 and 32 days after substrate amendment. Nevertheless, the microcosms were analysed by SIP only on days 8 and 32 for all substrates. In addition, samples from glucose microcosms were also analysed at day 2 because glucose is degraded rapidly and early degraders might already be missed at day 8 some. For cellulose, day 16 was also analysed, as labelled T-RFs differed profoundly between day 8 and 32 and it seemed important to monitor this transition. Fig. A.2 of the appendix contains T-RFLP fingerprints from all fractions of those RNA-SIP gradients. T-RFs were determined as labelled by comparison of abundances in "heavy" ^{13}C fractions with abundances in both "light" ^{13}C and "heavy" ^{12}C fractions. Labelled T-RFs and their assigned taxa are listed in Table 4.

Table 4: Labelled T-RFs in the detritusphere SIP experiment with assigned taxa. exp. T-RF: T-RF recognized as labelled by comparing T-RFLP fingerprint of SIP gradient fractions from ^{13}C treatments and ^{12}C-controls; *in-silico* T-RFs: best matching T-RF found in according contig sequences. n.d.: no distinct taxa were detected for this T-RF.

	day 8			day 32		
	exp. T-RF	taxa	*in-silico* T-RFs	exp. T-RF	taxa	*in-silico* T-RFs
glucose	61	*Arthrobacter*	67	61	*Arthrobacter*	67
	61	*uncl. Micrococcaceae*	67	61	*uncl. Micrococcaceae*	67
	71	*Arthrobacter*	68	137	*Nakamurella*	139
	80	*Flavobaterium*	85	145	*Humicoccus*	141
	159	*Arthrobacter*	161-163	490	*Pseudomonas*	490
	490	*Pseudomonas*	490-492			
cellulose	79	*Flavobacterium*	83	69	*uncl. Polyangiaceae*	76
	79	*Cytophaga*	82	69	*Sorangium*	76
	80	*Flavobacterium*	83,85	69	*Byssovorax*	76
	137	*Cellvibrio*	140, 143	80	*Flavobacterium*	85
	486	*Cellvibrio*	484, 486	84	*Flavobacterium*	85
	487	*Cellvibrio*	486, 487	157	*Kitasatospora*	81
	490	*Cellvibrio*	489-491	157	*uncl. Streptomyces*	159, 160
	524	n.d.		486	*Cellvibrio*	486
	535	n.d.		487	*Cellvibrio*	487
	583	n.d.		490	*Cellvibrio*	490
leaves	79	*Flavobacterium*	83	69	*uncl. Polyangiaceae*	76
	80	*Flavobacterium*	83,85	69	*Sorangium*	76
	84	*Flavobacterium*	83, 85, 86	80	*Byssovorax*	76
	89	n.d.		84	*Flavobacterium*	83
	93	n.d.		87	n.d.	
	205	*Ohtaekwangia*	207	109	n.d.	
	486	*Cellvibrio*	484, 487	205	*Ohtaekwangia*	207
	487	*Cellvibrio*	484, 488	487	*Cellvibrio*	488
	490	*uncl. Oxalobacteraceae*	489-491	490	*Cellvibrio*	490
	490	*Comamonadaceae*	490, 491	490	*uncl. Oxalobacteraceae*	490, 491
	524	*Mucilaginibacter*	523	500	*uncl. Polyangiaceae*	500-504
	535	n.d.		513	*uncl. Polyangiaceae*	515
	540	n.d.				
roots	77	*Flavobacterium*	83,85	69	*uncl. Polyangiaceae*	76
	79	*Flavobacterium*	83,86	69	*Byssovorax*	76
	80	*Flavobacterium*	83,87	69	*Sorangium*	76
	84	*Flavobacterium*	83,88	80	*Flavobacterium*	85
	486	*Cellvibrio*	484, 487	205	*Ohtaekwangia*	207
	487	*Cellvibrio*	484, 488	486	*Cellvibrio*	485, 486
	490	*uncl. Oxalobacteraceae*	490, 491	487	*Cellvibrio*	486, 487
	490	*Comamonadacea*	489, 490	490	*Cellvibrio*	489
	524	*Mucilaginibacter*	520	500	*uncl. Polyangiaceae*	502-505
				500	*Sorangium*	503
				500	*Byssovorax*	504

For glucose, identical labelled T-RFs were found after 2 and 8 days of substrate amendment: mainly bacteria with T-RFs of 61 bp (also: 71 bp, 80 bp and 490 bp) had apparently utilised glucose (Table 4). After 32 days, the glucose degrading population shifted: T-RFs of 61 bp and 137 bp were now dominant and the abundances of three more T-RFs (490 bp, 93 bp, 145 bp) were now increased in comparison to the control fractions. For cellulose, 8 T-RFs were clearly labelled after 8 days of incubation and no clear dominance of specific T-RFs was observed. After 32 days, however, bacteria with T-RFs of 69 bp and 157 bp appeared as the dominant cellulose degraders and 5 less abundant T-RFs (80 bp, 84 bp, 486 bp, 487 bp and 490 bp) also emerged (Table 4). The cellulose labelling pattern after 16 days represented a transition state, as labelled T-RFs both from day 8 and day 32 were present here with high abundances of the 157 bp T-RF.

Early leaf degraders appeared much more diverse and displayed 12 labelled T-RFs in total, with no clear dominance. After 32 days, 5 of these still appeared labelled (80 bp, 84 bp, 205 bp, 487, bp and 490 bp), but the leaf litter-degrading community partially shifted, and 5 additonal labelled T-RFs emerged (69 bp, 87 bp, 109 bp, 500 bp and 513 bp). T-RFs of root degraders were less diverse but generally similar to leaf degraders. After 8 days of inoculation, 8 T-RFs appeared labelled. After 32 days, a dominance of the 69 bp T-RF was observed and 7 additional T-RFs also appeared in 'heavy' rRNA (Table 4).

To compare the diversity of primary labelled populations across substrates, the functional organisation (*Fo*) of 'heavy' rRNA T-RFLP fingerprints was used (Marzorati *et al.* 2008). This indicator for the structural composition of a given community considers taxon richness and relative abundances, just as classical diversity indices like the Shannon-Wiener *H'*, but is more appropriate for fingerprinting data as rare taxa have less influence (Blackwood *et al.* 2007). The *Fo* index is formed by the cumulative abundance of the most dominant 20 % of all taxa, reflecting the overall distribution of taxa, and therefore, *Fo* increases with decreasing diversity and evenness (Wittebolle *et al.* 2008). *Fo* was applied to T-RFLP fingerprints of all 'heavy' ^{13}C rRNA fractions. Indeed, *Fo* was higher for labelled cellulose (day 8: 72, day 32: 82) and glucose degraders (day 8 and 32: 78) in comparison to labelled leaf (day 8: 69, day 32: 65) and root litter utilisers (day 8: 58, day 32: 71).

To identify labelled bacterial taxa, 16S rRNA of gradient fractions 3 ('heavy') and 8 ('light') from both ^{13}C and ^{12}C SIP gradients were sequenced. Sequences were again assembled into contigs to obtain full-length (~520 bp) amplicon sequences containing reads with both primers. Taxonomic identities were thus assigned to the distinctly labelled

T-RFs, wherever possible, according to matching *in-silico* and experimental T-RFs (Table 4). Furthermore, putative detritusphere substrate utilisers were again determined via pyrotag enrichment indicators (EI) calculated for each taxon. As substrate users were more abundant here compared to the rhizosphere experiment, taxa were defined as labelled from an enrichment indicator of >0.5 and a relative sequence abundance of 2 % in the "heavy" ^{13}C-rRNA fractions (Fig. 17, Fig. 18).

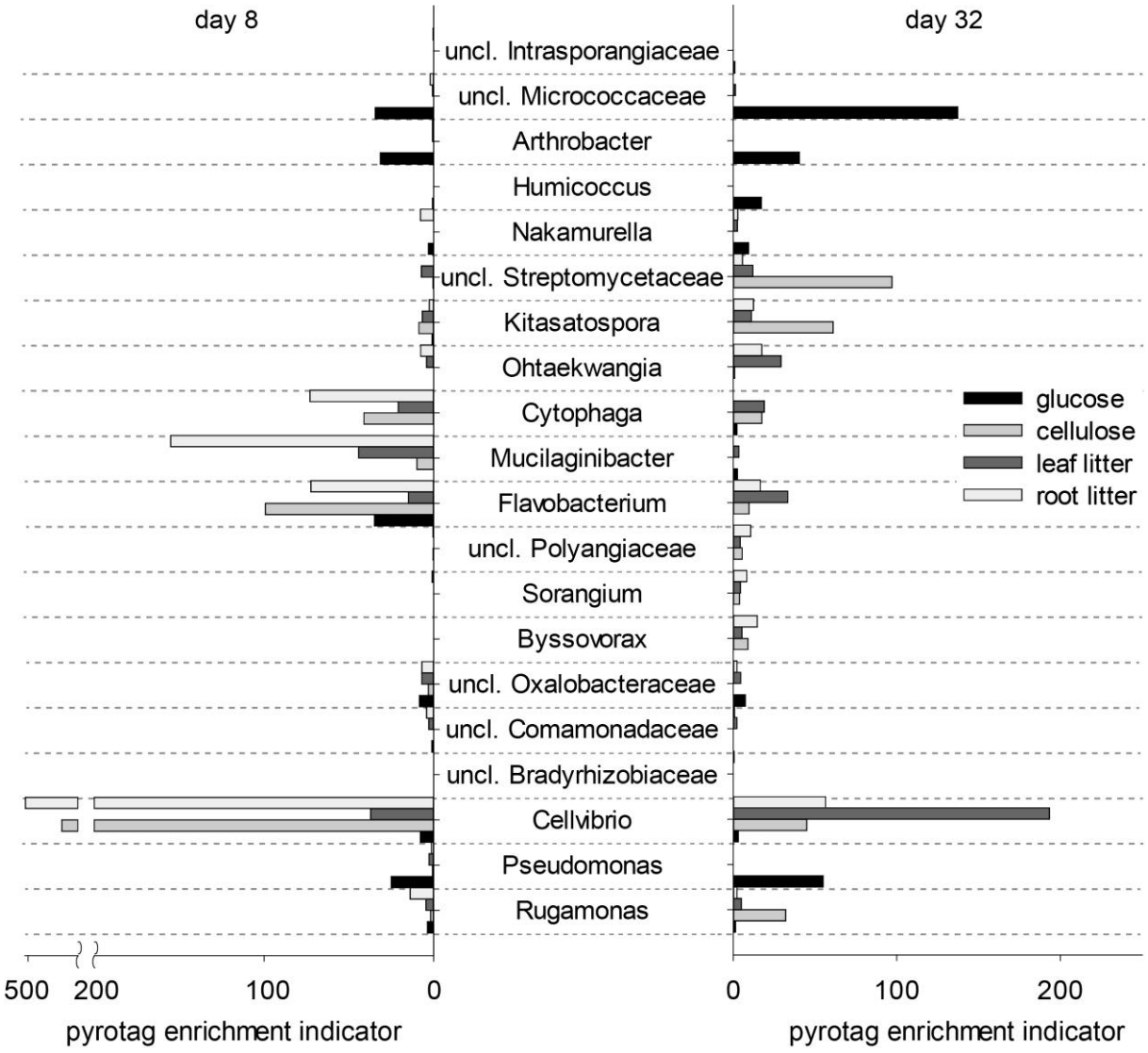

Fig. 17: Enrichment indicators of bacterial taxa labelled after consumption of ^{13}C-substrates at days 8 and 32 of the detritussphere SIP experiment. Taxa were defined labelled with enrichment indicator > 0.5 and > 2 % relative pyrotag abundance in 'heavy' rRNA.

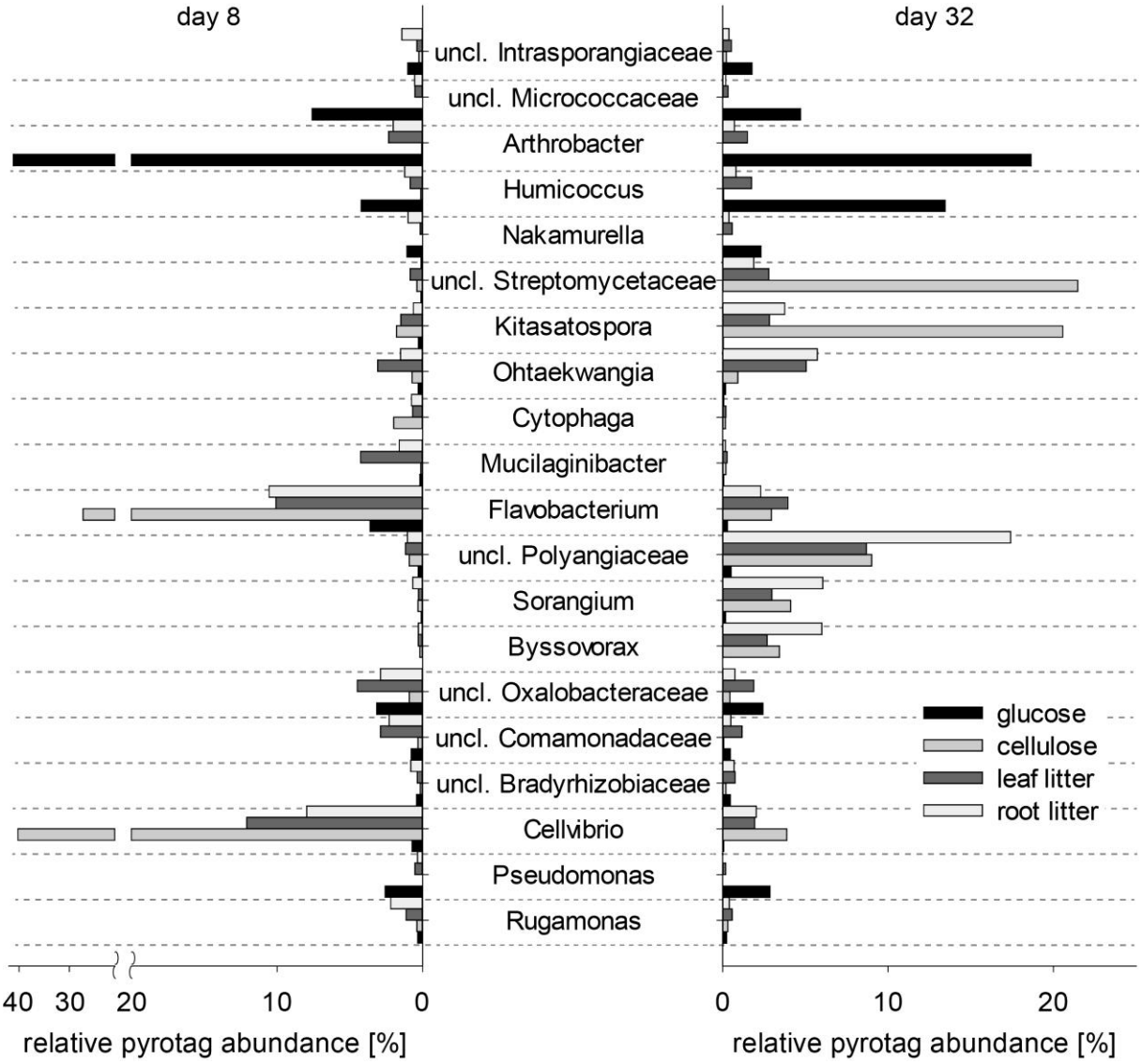

Fig. 18: Relative pyrotag abundance in "heavy" rRNA fractions of bacterial taxa labelled upon ^{13}C-substrate consumption at days 5 and 8 of the detritussphere SIP experiment. Taxa were defined labelled with enrichment factor > 0.5 and > 2 % relative pyrotag abundance in 'heavy' rRNA.

Glucose carbon was mostly assimilated by *Arthrobacter* spp. and later also by *Humicoccus* spp.. According to the fingerprinting results, no OTU dominated in the cellulose degrader population after 8 days, but with pyrosequencing a dominance of *Flavobacterium* and *Cellvibrio* spp. were revealed. This discrepancy is caused by the fact that *Cellvibrio* spp. featured several T-RFs (Table 4). Carbon from cellulose was also assimilated by *Cytophaga* spp. at day 8. At day 32, *Kitasatospora* spp. and other unaffiliated *Streptomycetaceae* were the dominant cellulose utilisers. Both leaf and root litter were degraded, amongst others, by *Flavobacterium* spp. and *Cellvibrio* ssp. at day 8, but also *Cytophaga* spp. and *Mucilaginibacter* spp. At the later time point, *Ohtaekwangia* spp. and members of the *Polyangiaceae* (*Sorangium* ssp., *Byssovorax* ssp. and unclassified

Polyangiaceae) were involved in carbon assimilation from litter substrates, too. In fact, degrader populations for leave and root litter were quite similar and very different to those utilising glucose. Some cellulose carbon utilisers, however, were also found to be important for litter degradation (*Kitasatospora* ssp., *Cellvibrio* ssp., *Polyangiaceae* ssp.). Distinct activity and growth patterns were observed for some bacteria: *Flavobacterium* spp. and *Mucilaginibacter* ssp. always appeared to utilise the substrates at an early stage (8 days), whereas the activity of *Streptomycetaceae*, *Polyangiaceae* and *Ohtaekwangia* ssp. was delayed (32 days). It was apparent that some genera were highly enriched but not very abundant in heavy rRNA, *e.g.* species of the genera *Cytophaga*, *Pseudomonas* and *Mucilaginibacter*. Partially, no labelled T-RF could be assigned to them. Nevertheless, I could clearly assign all of the more abundant ^{13}C-enriched taxa to labelled T-RFs. The overall degradation patterns suggested by the labelled T-RFs was largely confirmed by the relative abundances of labelled taxa in pyrotaq sequencing: glucose and cellulose were mainly degraded by one or two dominant and highly abundant taxa, whereas the more complex and natural substrates, leaf and root litter, were utilised by a more diverse population without explicit dominance.

3.2.3. Bacterial biomass SIP experiment

This experiment was conducted to obtain further insights into the carbon flow within soil microbiota, starting from ^{13}C-labelled biomass of putative root exudate consumers. Here, I wanted to identify saprophytic and predatory secondary bacterial links within the soil food web. Therefore, putative root exudate utilising bacteria were enriched in liquid medium from the field soil. They were labelled by growth on an artificial ^{13}C-root exudate mixture at natural concentrations (Marx *et al.* 2010). ^{12}C controls were generated in the same way. The enrichment cultures were analysed by T-RFLP fingerprinting and amplicon sequencing. The majority from both the ^{12}C and the ^{13}C culture were affiliated to *Cupriavidus* ssp. (TRF: 429 bp), *Pseudomonas* ssp. (490 bp), *Burkholderia* ssp. (139 bp) and unclassified *Burkholderiaceae* (490 bp). These enriched and labelled root exudate degraders (93.64 % ^{13}C as measured by CHN analyser) were added in drops to sieved field soil. After amendment, the soil was mixed thoroughly and distributed to the respective microcosms. SIP and subsequent T-RFLP fingerprinting of rRNA gradient fractions was done for the samples taken after 1, 2, 3, 8 and 12 days of incubation and the respective "heavy" RNA fraction number 2 were sequenced.

Table 5: Labelled T-RFs from the biomass experiment with assigned taxa. Bold letters indicate initially added bacteria. T-RFs in parentesesare secondary and tertiary enzyme restriction sites. Exp. T-RF: T-RF recognized as labelled by comparing T-RFLP fingerprint of SIP gradient fractions from ^{13}C treatments and ^{12}C-controls; *in-silico* T-RFs: best matching T-RF found in according contig sequences.

day 1			**day 3**		
exp. T-RF	taxa	*in-silico* T-RFs	exp. T-RF	taxa	*in-silico* T-RFs
139	***Burkholderia***	141	**139**	***Burkholderia***	141
149	***Cupriavidus***	(147)	139	*Marmoricola*	141
429	***Cupriavidus***	430	139	*Humicoccus*	141
487	*Diaphorobacter*	488	**149**	***Cupriavidus***	(147)
490	***Pseudomomnas***	490	**429**	***Cupriavidus***	430
492	*Acinetobacter*	491	487	*Diaphorobacter*	488
			490	***Pseudomomnas***	490
			492	*Pseudomomnas*	492
day 8			**day 12**		
exp. T-RF	taxa	*in-silico* T-RFs	exp. T-RF	taxa	*in-silico* T-RFs
139	***Burkholderia***	141	**139**	***Burkholderia***	141
139	*several Actinobacteria*	139-142	426	*Cupriavidus*	428
426	*Diaphorobacter*	428	**429**	***Cupriavidus***	430
429	***Cupriavidus***	429	**456**	***Burkholderia***	(456)
456	***Burkholderia***	(456)	**476**	***Cupriavidus***	(476)
476	***Cupriavidus***	(476)	**490**	***Pseudomomnas***	490
490	***Pseudomomnas***	490			

Throughout the experiment, a large part of the added bacteria remained unchanged. After 12 days, the "heavy" fractions of the ^{13}C treatment were still dominated by T-RFs of 429 bp and 490 bp (Table 5, appendix Fig. A.3) and their respective bacterial taxa (Fig. 19). Therefore, most of the initial 'bait' biomass carbon appeared to not have entered the food web, and secondary consumers were hardly found. Nevertheless, both, T-RFLP fingerprinting and pyrotaq sequencing revealed that already after one day of inoculation, secondary consumers were detectable: *Diaphorobacter* spp. (TRF: 487 bp) and *Acinetobacter* spp. (492 bp) appeared labelled in addition to the original inoculum. After three days, *Marmoricola* spp. and *Nocardioides* spp. (both *Actinobacteria*) were also labelled, which were both contributing to the elevated abundance of the labelled T-RF of 139 bp. Aditionally, *Singulisphaera* ssp. and other unaffiliated *Planctomycetaceae* exhibited ^{13}C enriched RNA, but their abundance was too low to be apparent in fingerprints. On day 8, already fewer secondary degraders were detectable and after 13 days only the initially added bacteria remained still clearly enriched in "heavy" fractions (Table 5, Fig. 19).

The labelled T-RFs of 149 bp (day 1 and day 2), 456 bp and 476 bp (day 8 and 12) could not be assigned to any taxa, as no contigs with these *in-silico* T-RF were found. However, the second and third *Msp*I restriction site of the *Cupriavidus* spp. 16S rRNA gene are linked to the 147 bp and 476 bp T-RFs, and the second restriction site for *Burkholderia* spp. contigs was at 456 bp.

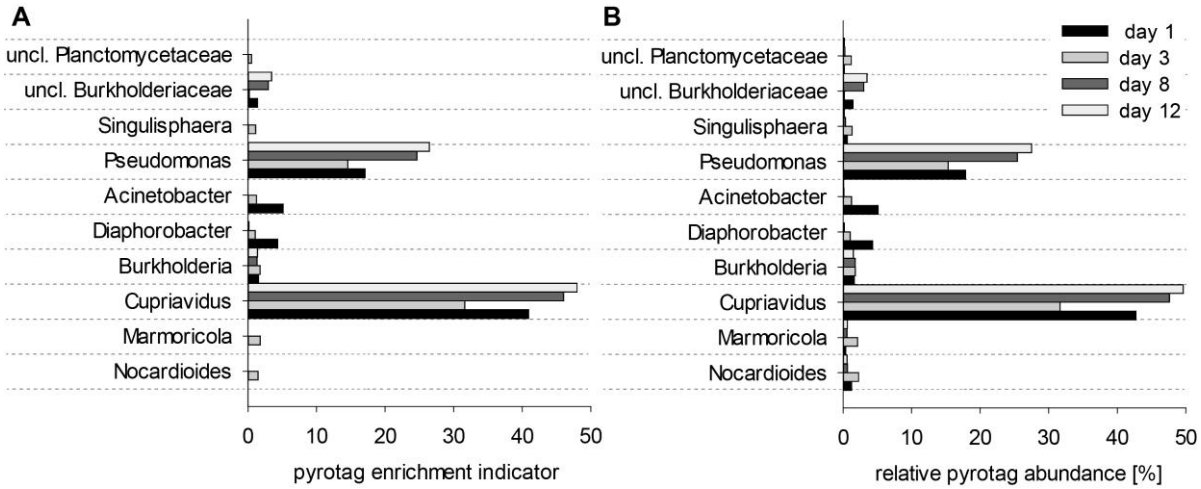

Fig. 19: Enrichment (A) and relative sequence abundance (B) in "heavy" rRNA fractions of bacterial taxa labelled upon ^{13}C-biomass consumption in the biomass SIP experiment. Taxa were defined labelled with enrichment factor > 0.5 and > 2 % relative pyrotag abundance in 'heavy' rRNA.

3.2.4. Synthesis of SIP experiments

Three labelling experiments were carried out in this thesis, partly together with other Research Unit members. To cover the most important carbon sources for soil microbiota, rhizodeposits, litter (and single litter compounds), as well as bacterial biomass itself, were used as ^{13}C-sources. Rhizodeposits were used by a very diverse bacterial community. For example *Mucilaginibacter* spp., *Massilia* spp. and *Opitutus* spp. were important here. Glucose was mainly degraded by *Micrococcaceae*. Central cellulose degraders were *Cellvibrio* spp., *Flavobacterium* spp., and *Streptomycetaceae.* Maize root and leaf litter degraders were more diverse and here, *Cytophaga* spp., *Mucilaginibacter* spp., *Cellvibrio* spp. and also *Polyangiaceae* were very important degraders (Table 6). Added ^{13}C enriched bacteria in the biomass labelling experiment remained mostly intact, and only little ^{13}C carbon was transferred to secondary degrader populations. Biomass carbon was incorporated, amongst others, by *Acinetobacter* spp. and *Diaphorobacter* spp..

Table 6: Most important taxa identified in the 3 labelling experiments and the substrates they incorporated mostly.

	taxa	substrate
A*ctinobacteria*	*Arthrobacter*	glucose, (rhizodeposits)
	Humicoccus	glucose
	Kitasatospora	cellulose, rhizodeposits
	uncl. *Streptomycetaceae*	cellulose
	uncl. *Micrococcaceae*	glucose
Bacteroidetes	*Cytophaga*	cellulose, litter
	Flavobacterium	(glucose), cellulose, litter
	Mucilaginibacter	litter, rhizodeposits
	Ohtaekwangia	rhizodeposits, litter
Alphaproteobacteria	*Sphingobium*	rhizodeposits
Betaproteobacteria	*Diaphorobacter*	biomass
	Massilia	rhizodeposits
Gammaproteobacteria	*Acinetobacter*	biomass
	Cellvibrio	cellulose, litter
	Pseudomonas	glucose
Deltaproteobacteria	*Byssovorax*	cellulose, litter, (biomass?)
	Sorangium	cellulose, litter, (biomass?)
	uncl. *Polyangiaceae*	cellulose, litter, (biomass?)
Verrucomicrobia	*Opitutus*	rhizodeposits

The different substrates were largely degraded by distinct bacterial populations with some overlaps: Root and leaf litter degraders were almost identical and very similar to cellulose utilisers. Besides, some rhizodeposit consumers appeared to have incorporated carbon also from glucose (*Athrobacter* spp.) and cellulose (*Kitasatospora* spp., *Mucilaginibacter* spp.). Apart from substrate specificity, different lag phases were observed for carbon use, indicating copiotrophic and oligotrophic life styles. *Polyangiaceae* and *Streptomycetaceae*, for example, were always late degraders, whereas *Cellvibrio* and *Flavobacterium* mostly incorporated ^{13}C at the beginning of the experiments. Some taxa were highly enriched but not very abundant, indicating pronounced substrate specificity. Sometimes, ^{13}C substrates were only a part of the carbon sources used by very abundant taxa causing a relatively low enrichment factors.

3.3. Back to the field: identified bacterial food web in their natural habitat

The bacteria identified in the different SIP microcosm experiments, were now localised in the field. Thus, the natural distributions and abundances of key food web bacteria were to be evaluated by a combination of amplicon sequencing and qPCR. Bulk soil samples from all depths and sampling time points of a plot with corn maize cultivation (shoot litter added

after harvest) were analysed by bacterial 16S pyrotag sequencing. In addition, rhizosphere and root surface samples were sequenced from July and December 2009.

3.3.1. Overview of bacterial taxa in the field

For an overview of the distribution of all bacterial taxa in the field soil, relative sequence abundances of phyla were summarised in Fig. 20. At the phylum level, *Proteobacteria* clearly constitute the most abundant group. They represented ~ 30 % of all bacterial sequences in bulk soil samples, and their ratio further increased in the rhizosphere (~ 35 %) and on root surface samples (~ 55 %). *Alphaproteobacteria* were the predominant *Proteobacteria* in the bulk soil and rhizosphere, and *Betaproteobacteria* became equally abundant at the root surface. *Betaproteobacteria* tended to increase, whereas *Alphaproteobacteria* and *Gammaproteobacteria* declined slightly with depth. Sequences affiliated to *Bacteroidetes* increased likewise in the rhizosphere and at root surface, and also decreased with depth.

Acidobacteria and *Firmicutes*, on the contrary, were much more frequent in the bulk soil (~ 15 % and ~12 %) than at the root surface (~ 2 % and ~ 1 %). In the rhizosphere, they were of lower abundance in winter (both ~ 6 %) than in summer (both ~ 14 %).

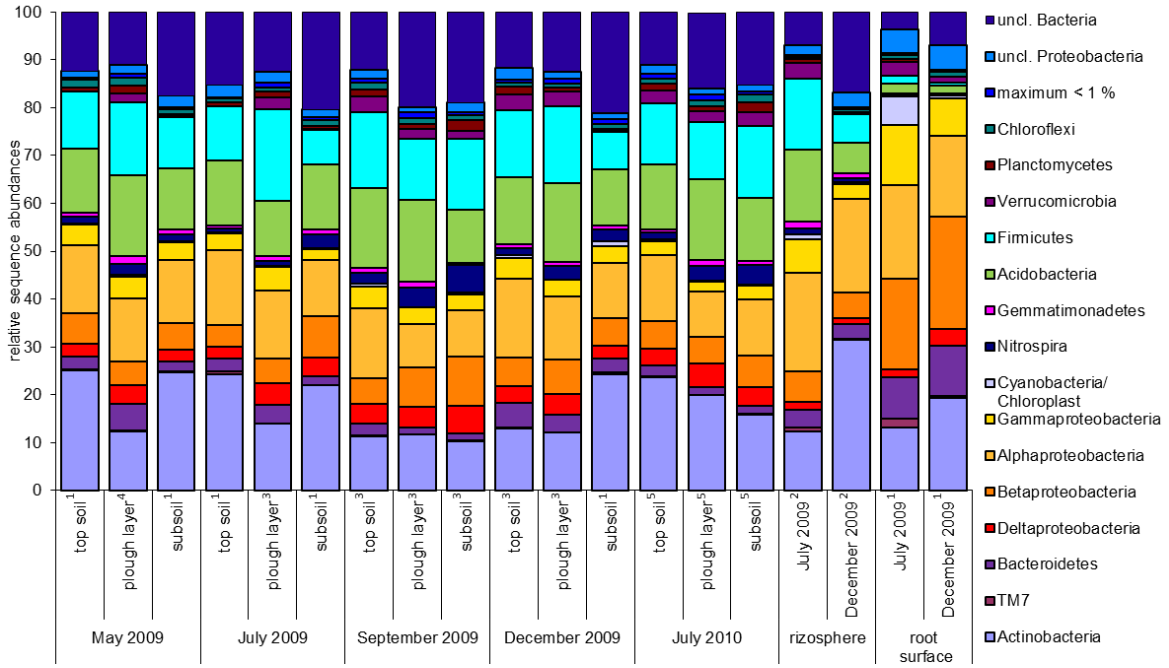

Fig. 20: relative sequence abundances of the most important phyla (classes for *Proteobacteria*) in the field. Identical superscript symbols (*, ~, ° and ‘) indicate samples sequenced in the same 454 pyrosequencing run.

The abundance of *Actinobacteria* varied strongly for the bulk soil samples, and no correlation to natural parameters was found (Fig. A.4) Relative sequence abundances of *Actinobacteria* were not as reproducible between different sequencing runs as previously observed (Pilloni *et al.* 2012a). Possibly, this may have been related to a change in the 454 sequencing chemistry, or to general problems of sequencing bacteria with high G+C % content, which will be discussed further down. However, experimentally identified *Actinobacteria* (*Arthrobacter* spp., *Humicoccus* spp, *Kitasatospora* spp., unclassified *Micrococcaceae* and unclassified *Streptomycetaceae*) were hardly affected (Fig. 21).

Species affiliated to *Massilia*, *Mucilaginibacter*, *Pseudomonas*, *Streptomyces* and several rhizobacteria (*Rhizobium*, *Mesorhizobium*, *Bradyrhizobiaceae*) were among the most abundant taxa (> 1 % of total read,) from the maize root surface and rhizosphere (appendix Fig. A.5). Especially the assigned T-RFs of *Peudomonas* spp. (490 bp), *Massilia* spp. (487 bp), and *Mucilaginibacter* spp. (435 bp) were also determining the grouping of root surface and rhizosphere samples apart from bulk soil samples in the PCA for all T-RFLP fingerprinting data (Fig. 11). At root surface also bacteria from *Dokdonella*, *Duganella*, *Niastella*, *Opitutus* and *Ohtaekwangia* were abundant. In rhizosphere, however, also many typical top soil genera were abundant like *Bacillus* spp., *Arthrobacter* spp. and bacteria affiliated to *Acidobacteria* subgroup 6. In bulk soil, some taxa were only found in top soil (*Marmoricola* spp. and *Nocardioides* spp), and others decreased with depth (*Rhizobiales*, *Acidobacteria* subgroup 4). The plough sole was preferred by unclassified *Deltaproteobacteria* and *Acidobacteria* subgroup 6 and in subsoil *Nitrospira* spp. was most abundant. Generally, classifiable sequences were highest at root surface and in rhizosphere and decreased with depth in bulk soil. Especially reads from unclassified *Actinobacteria* and unclassified bacteria were highest in subsoil.

3.3.2. Distribution of identified bacterial food web members

Relative sequence abundances of the experimentally identified key bacterial food web taxa were extracted from the pyrosequencing data set. In fact, reads from experimental rhizodeposit users were exceeding in the field rhizosphere, especially at the root surface and in summer, due to the high pyrotag abundances of especially *Massilia*, *Mucilaginibacter* but also *Opitutus* and *Ohtaekwangia* spp. (Fig. 21). Reads of experimentally identified detritussphere substrate utilisers were also most abundant at the root surface, but in contrast to rhizodeposit utilisers they were also abundant in bulk soil. In July, *Pseudomonas* (glucose degrader and PGPR) and *Mucilaginibacter* spp. (also

identified rhizodeposit utiliser) were abundant on the root surface. In December, *Flavobacterium* spp. (all substrates), *Ohtaekwangia* spp. (litter and rhizodeposits) but also *Mucilaginibacter* spp. and unclassified *Polyangiaceae* were of marked abundance on decaying roots. In bulk soil, bacteria affiliated to *Arthrobacter*, unclassified *Micrococcaceae*, *Flavobacterium* but also *Pseudomonas* and *Humicoccus* were the most abundant of the identified detritussphere substrate utilisers. Pronounced depth dependence or seasonal changes were not observed. *Cytophaga* spp., *Cellvibrio* spp. and unclassified *Streptomycetaceae* were generally of low abundance in the field, although they were highly enriched at early time points in the SIP experiments with cellulose and litter.

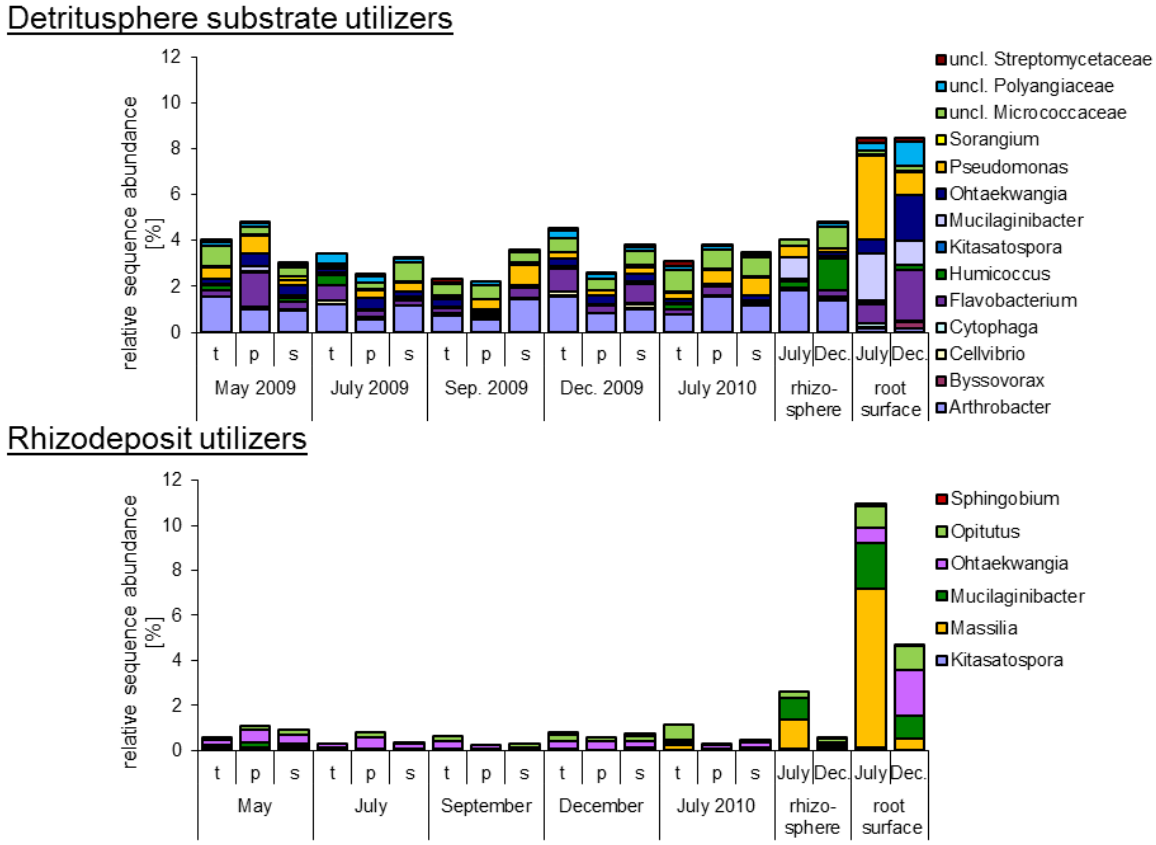

Fig. 21: relative pyrosequencing read abundances from the field samples of the most important experimentally identified key food web bacteria from rhizodeposit and substrate SIP experiments. t = top soil, p = plough sole, s = subsoil

Beside these distributional heterogeneities based on read abundances, cell numbers of identified key taxa were deduced from amplicon pyrosequencing and qPCR data. As 16S rRNA gene copy numbers can vary greatly among bacterial taxa, read abundance may not be sufficient to approximate specific biomasses. Generally, cell numbers are much more appropriate for conceptual food web models, as they are decisive for carbon turnover.

Table 7: Average 16S RNA gene copies per cell (c/c) derived from all taxa specific genomes available at the IMG data base (Markowitz *et al.* 2012).

taxa	c/c
Acinetobacter	3.30
Arthrobacter	3.46
Byssovorax	4.00
Cellvibrio	3.00
Cytophaga	3.33
Diaphorobacter	2.80
Flavobacterium	2.63
Humicoccus	2.00
Kitasatospora	9.00
Massilia	1.33
Mucilaginibacter	4.00
Ohtaekwangia	2.76
Opitutus	1.00
Pseudomonas	2.71
Sorangium	4.00
Sphingobium	2.60
uncl. *Streptomycetaceae*	3.88
uncl. *Micrococcaceae*	2.70
uncl. *Polyangiaceae*	4.00

Cell numbers were calculated from relative read abundances of identified key taxa which were multiplied with absolute 16S RNA gene counts from qPCR analysis of the same soil (Fig. 14) and divided by taxon specific operon copy numbers per cell (Table 7).

These cell number estimates are plotted in Fig. 22 for substrate-defined groups of the most important identified taxa from the SIP experiments (Table 6). Generally, consumer cell numbers clearly decreased with depth and were most abundant in the rhizosphere samples. Cell numbers of identified taxa appeared to be not much affected by seasonal changes in bulk soil samples, but the rhizosphere generally comprised much higher abundances in July compared to December. For root surface samples, the opposite was true, mostly due to the much higher overall 16S operon abundance from qPCR analysis in winter.

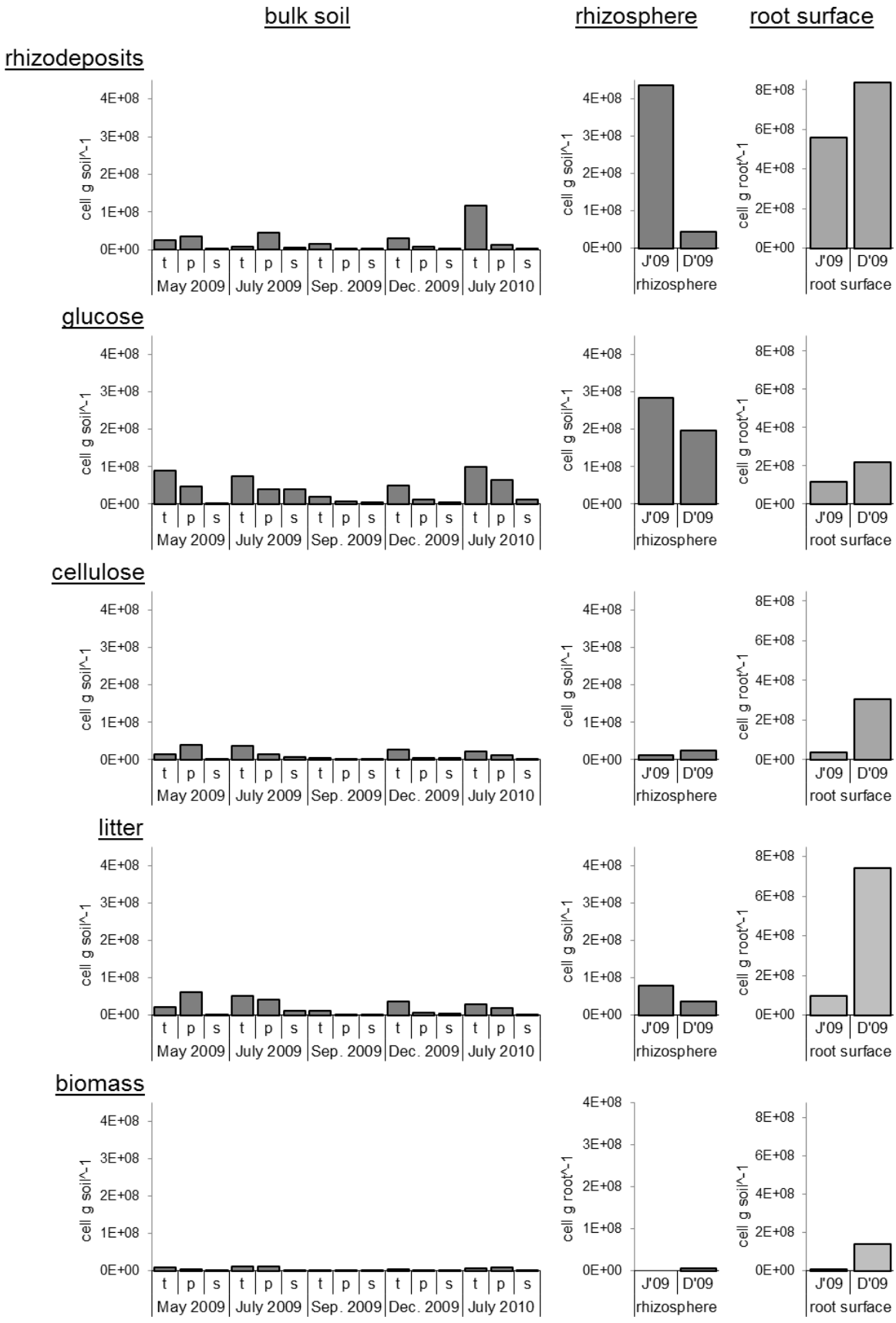

Fig. 22: Abundances of experimentally identified groups of key bacterial taxa potentially involved in the consumption of specified substrates in the field. Bulk soil and rhizosphere cell numbers are related to g soil, root surface cell numbers to g root.

Comparing identified utilisers of the different experiments and substrates, potential glucose and rhizodeposit users had highest and biomass degraders lowest abundances in the field. Putative litter and cellulose degraders were of modest frequency. Bacteria identified to assimilate carbon from rhizodeposits and glucose were more frequent in rhizosphere than in bulk soil and abundances of cellulose, litter and biomass degraders were similar for rhizosphere and bulk top soil. In bulk soil, glucose utilisers were most abundant and for all substrates, abundances of the most important utilisers (Table 7) were lowest in subsoil and highest in top soil with some exceptions where the plough sole layer contained highest cell numbers of respective identified food web members. No distinct seasonal influence on cell numbers of identified bacteria was observed in bulk soil but in rhizosphere, numbers of rhizodeposit utilisers in July exceeded those in December by far. Degrease in rhizosphere was also observed for glucose and litter degraders and cellulose degraders increased slightly in winter. For root surfaces, the abundances of potential bacterial food web members were always highest in December. This difference was most prominent for identified litter and cellulose degraders, where also sequencing read numbers showed the same pattern. Cell numbers of rhizodeposit and glucose utilisers were also higher in winter at root surface although their relative 16S rRNA gene numbers exceeded in July but the difference was less pronounced than for utilisers of other substrates. This was mostly due to very high overall 16S rRNA gene abundance in December compared to July.

In summary, identified key bacterial taxa putatively involved in the soil food web were not distributed evenly in the field. The rhizosphere comprised all in all much more of the relevant bacteria than bulk soil, and taxa composition was different here compared to bulk soil. Seasonal changes were mostly observed in the rhizosphere and at the root surface.

3.4. Mobilisation and transport of soil bacteria by seepage water

The influence of transported bacteria to deeper soil layers on food webs and carbon flow has been neglected so far. Therefore here, natural bacterial communities mobilised by seepage water in the agricultural soil were investigated. I sampled fresh lysimeter water over 24 h after an event of snowmelt and rain in January 2011. This was the minimal time span needed to obtain enough seepage water (50 and 75 ml) at the depths of 38 cm (L35) and 65 cm (L65) respectively, where lysimeters were installed. In this time span ‘bottle effects’ of sampled seepage water were probably avoided. Lysimeters were installed and maintained by the group of K.U. Totsche of the University of Jena. Corresponding bulk soil near the lysimeters was sampled by coring at 0-10 cm (B10), 40-50 cm (B50) and 60-

70 cm (B70) on the same day. Additionally, rhizosphere and root samples from a remaining capped maize stalk were also sampled. Due to the very limited amount of available seepage water, replicate sampling was not possible, and technical replication was applied for lysimeter DNA extracts. For soil and root samples, biological replication was done, of course.

3.4.1. Bacterial community patterns in seepage water and soil

T-RFLP and sequencing data sets were first screened with principal component analysis (PCA) to reduce complexity and allow overall comparisons. For both analysing methods, PCA arranged the samples in a very similar pattern (Fig. 23). Lysimeter samples grouped together and were discriminated from the bulk soil communities by a negative PC1, together with the root surface samples. PC1 accounted for 37 % of overall T-RF variability and 60 % of overall 16S rRNA gene sequence abundance variability. Rhizosphere samples were placed closest to the 10 cm bulk soil samples in the PCA biplots, but with a slight tendency towards root surface samples. While PC1 seemed mostly influenced by the sampled compartment, PC2 appeared predominantly controlled by depth. In general, triplicate fingerprints from technical (water) or biological (soil, roots) replicates were well comparable as visualised by PCA of T-RFLP fingerprints (Fig. 23a). Only one replicate of the 40-50 cm bulk soil extracts appeared more similar to the 0-10 cm bulk soil samples than the other two replicates.

PCA biplots also depicted T-RFs and bacterial taxa identified in pyrotag sequencing as vectors with their impact on sample ordination. The bacterial taxa (Fig. 23b) representative for lysimeter water were mostly within the *Betaproteobacteria* (*e.g. Methylophilaceae*, *Oxalobacteraceae* and *Comamonadaceae*), but also the *Alphaproteobacteria* (*e.g. Sphingobacteriaceae* and *Bradyrhizobiaceae*), *Gammaproteobacteria* (*e.g. Legionellaceae*) and *Bacteroidetes* (*e.g. Sphingobacteriaceae*). In contrast, reads affiliated to *Acidobacteria* (*e.g.* the subgroups 1, 4, 6, and 7 (Jones *et al.* 2009), *Firmicutes* (*e.g. Bacillaceae*, *Paenibacillaceae*) and *Actinobacteria* (*e.g. Nocardiaceae* and *Micromonosporaceae*) were clearly more abundant in bulk soil compartments (Fig. 23b).

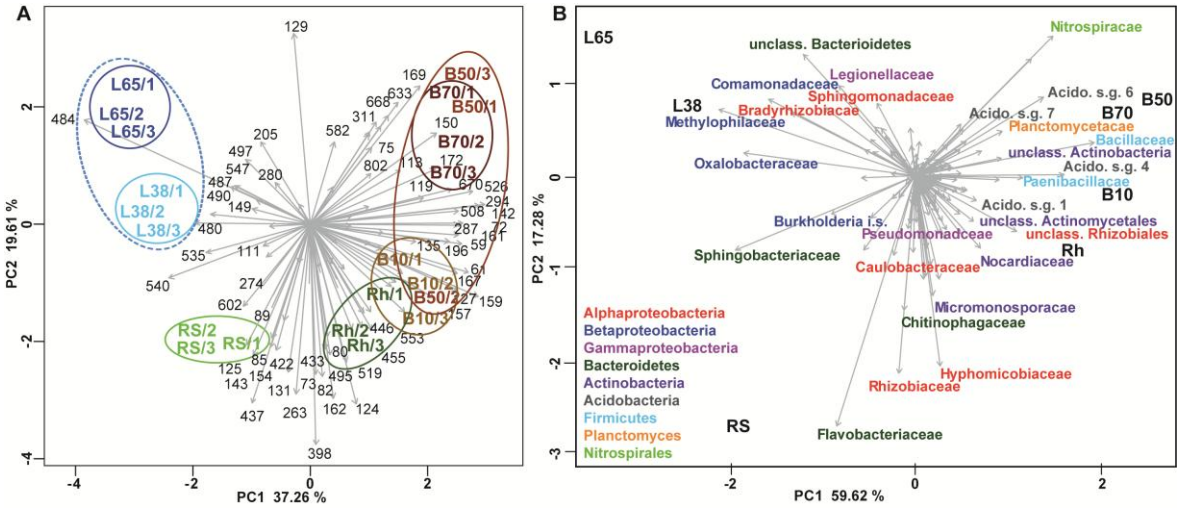

Fig. 23: PCA biplots for the T-RFLP (in bp, A) and amplicon sequencing (B) datasets. Samples are written in bold letters, important species in regular. All T-RFs (A) and taxa (B) are denoted by grey arrows but only central species are designated. Variance explanation proportions for each principal component (PC) are given in brackets. L38, L65: lysimeter samples from 38 cm and 65 cm depth; B10, B38, B65: bulk soil samples from 0-10, 40-50 and 60-70 cm depth; Rh: rhizosphere; RS: root surface

To further support linking of both T-RF and pyrotag data sets, I aimed to match some of the identified discriminative T-RFs to assembled dominating amplicon contigs from the same samples (Pilloni *et al.* 2012a). Although not unambiguous for all relevant T-RFs, consistencies between both approaches were revealed. Hence, I could observe that a majority of the *Bradyrhizobiaceae*- and *Sphingomonadaceae*-related reads were assigned to the T-RF of 149 bp and most members of the *Comamonadaceae* and *Moraxellaceae* comprised a fragment of 484 bp. *Legionellaceae* could be assigned to T-RFs of 490 bp and 497 bp and *Methylophilaceae*, *Oxalobacteraceae* and *Pseudomonadaceae* to a fragment of 490 bp. All of these T-RFs and lineages were clearly characteristic for lysimeter samples. Moreover, reads within the *Chitinophagaceae*, also typical for root surface samples, were matched to T-RFs of 85 bp and 89 bp and both TRFs tended towards root surface samples in the PCA biplot. Also, several typical bulk soil taxa were assigned to T-RFs frequent in the respective samples: *Bacillaceae:* 150 bp*; Paenibacillaceae*: 161 bp, *Acidobacteria*: subgroup 4*:* 142 bp; subgroup 6*:* 294 bp; subgroup 7: 142 bp*; Nocardiaceae:* 135 bp and *Micromonosporaceae*: 159 bp (Fig. 23).

3.4.2. Selective mobilisation and transport of bacterial lineages

As bacteria from the lysimeter samples were clearly not a representative subset of total soil bacterial communities, a selective mobilisation and transport was suggested. In fact,

relative sequence abundances of overall phyla differed profoundly between lysimeter water and soil samples (Fig. 24). As mentioned above, also the taxa most prominently mobilized by seepage water were not likewise abundant in bulk soil samples. Most prominently, Gram-positive bacteria like *Firmicutes* and *Actinobacteria* were abundant in bulk soil and rhizosphere (8 -16 % read abundance), but almost absent in Lysimeter samples (below 1 %, Fig. 24). Instead, many taxa abundant in lysimeter water were also frequent on the root surface, *e.g.* the *Oxalobacteraceae* (L38: 10 %, L65: 12 %, RS: 8 %), *Comamonadaceae* (L38: 4%, L65: 17 %, RS: 7 %) and *Sphingobacteriaceae* (L38: 7 %, L65: 6 %, RS: 7 %). For other lineages, however, relative sequence abundances were low for all soil and root samples, and relatively high in lysimeter water: *e.g.* the *Opitutaceae* (L38: 3 %), *Bradyrhizobiaceae* (L38: 2 %, L65: 13 %), *Sphingomonadaceae* (L38: 4 %, L65: 7 %), *Methylophilaceaea* (L38: 6 %, L65: 10 %) and *Legionallaceae* (L38: 3 %, L65: 2 %). Finally, different taxa appeared distinctly abundant in seepage water at the two depths. *E.g.* reads of the *Gammaproteobacteria* were much more abundant at 35 cm (20 %) than at 65 cm (7 %), whereas *Alpha-* and *Betaproteobacteria* were more frequent in lysimeter water at 65 cm (*Alphapr.*: 23 %, *Betapr.*: 45 %) than at 35 cm depth (*Alphapr.*: 11 %, *Betapr.*: 24 %).

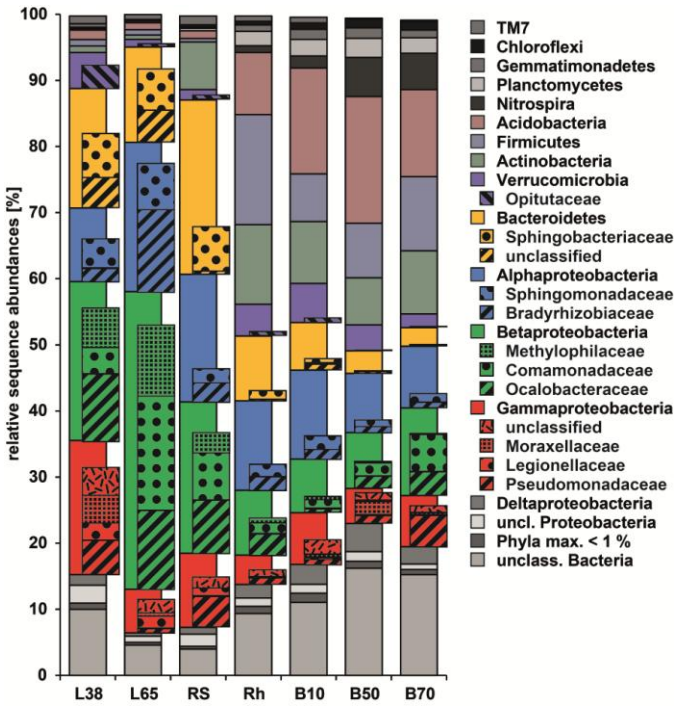

Fig. 24: Relative sequence abundances for all phyla obtained by amplicon sequencing. Highly mobilised taxa are highlighted and shaded. Abbreviations as in Fig. 23

3.4.3. Bacterial contribution to mobilised carbon

Total organic carbon (TOC) and dissolved organic carbon (DOC) were measured in seepage water and particulate organic carbon (POC) was calculated from those values (Table 8). Carbon measurements were all conducted by Andreas Schmalwasser of K.U. Totsches' group. Overall, we observed a TOC transport by seepage water of 1.1 g C m^{-2} below the plough horizon and 0.8 g C m^{-2} below the main root zone in the hydraulic year 2011. The fraction of POC was 6 % (0.07 g C m^{-2}) for 35 cm and 14 % (0.10 g C m^{-2}) for 65 cm depth.

Table 8: Numbers estimated for all samples to determine bacterial carbon proportion on washed-out organic carbon. Abbreviations as in Fig. 23

Sample	**L38** u = ml	**L65** u = ml	**B10** u = g	**B50** u = g	**B70** u = g
16s RNA gene abundance qPCR [copies u^{-1}]	$1.20*10^5$ ± $5.04*10^4$	$5.50*10^4$ ± $1.75*10^4$	$5.50*10^9$ ± $1.99*10^9$	$8.54*10^8$ ± $3.34*10^8$	$6.52*10^8$ ± $2.91*10^8$
average *rrn* copies per cell weighted mean of families [a]	2.69	2.47	2.67	2.67	2.96
bacterial cell number copies / copies per cell [cells u^{-1}]	$4.48*10^4$ ± $1.87*10^4$	$2.23*10^4$ ±$7.10*10^3$	$2.06*10^9$ ± $7.44*10^8$	$3.20*10^8$ ± $1.25*10^8$	$2.20*10^7$ ± $9.84*10^7$
bacterial biomass carbon cell number * 26.27 fg carbon $cell^{-1}$ [µg C u^{-1}]	$1.18*10^{-3}$ ± $4.92*10^{-4}$	$5.85*10^{-4}$ ± $1.86*10^4$	$5.41*10^1$ ± $1.96*10^1$	8.4 ± 3.28	5.78 ± 2.58
microbial biomass carbon measured for soil samples [µg Cu^{-1}]	--	--	$1.24*10^2$ [b]	$1.11*10^1$ [b]	9.86 [b]
bacterial biomass carbon 40 – 85 % of microbial carbon [µg C u^{-1}]	--	--	$4.96*10^1$ – $1.06*10^2$	4.42 – 9.4	3.94 – 8.38

[a] Taken from the IMG database (Markowitz et al., 2012).
[b] Taken from (Kramer *et al.* 2012)

The contribution of bacterial biomass carbon to this efflux was estimated based on 16S RNA gene abundances quantified in seepage water by quantitative PCR. Varying 16S rRNA gene copy numbers for different bacterial species were taken into account. Average 16S rRNA gene copy numbers for important bacterial taxa of each sample (appendix Table A.4), again deduced from the integrated microbial genome database (IMG, Markowitz *et al.* 2012), were weighted according to their abundances resulting in a weighted mean operon copy number for every sample. Thus, for lysimeter waters, an average of 2.69 (L35) and 2.47 (L65) 16S rRNA gene copy numbers per cell were deduced (Table 9).

Table 9: Organic carbon contents and numbers of estimated bacterial carbon proportions to washed-out organic carbon. Abbreviations as in Fig. 23.

Sample	**L35** u=ml	**L65** u=ml	**B10** u=g soil	**B50** u=g soil	**B70** u=g soil
TOC(OC > 0.45 µm) [µg u^{-1}]	5.13 ± 0.27	5.97 ± 0.13	$11.3*10^3$	$10.3*10^3$	$4.4*10^3$
DOC (OC < 0.45 µm) [µg u^{-1}]	4.77 ± 0.08	5.53 ± 0.15	--	--	--
OC< 0.2µm [µg u^{-1}]	2.87 ± 0.74	3.67 ± 0,54	--	--	--
Bacterial contribution to TOC [%]	0.02	0.01	0.48	0.08	0.13
Bacterial contribution to DOC [%]	0.27	0.10	--	--	--
Bacterial contribution to OC > 0.2 µm [%]	0.04	0.02	--	--	--

From this, together with estimated cellular abundances, mobilised bacterial carbon per water sample was inferred with an assumption of 26.27 fg carbon per cell (Troussellier *et al.* 1997). I also compared our calculations for bacterial carbon from soil samples with the measured microbial biomass carbon from the same soils (Kramer *et al.* 2012). Considering that between 40 % and 85 % of total microbial biomass carbon is considered to be of bacterial origin, both estimates were in the same order of magnitude (Table 8). According to these estimates, only 0.01 % to 0.02 % of total organic carbon mobilised in seepage water upon snowmelt related seepage events originated from actual bacterial cells, a ratio appearing exceedingly low (Table 9) at first site.

4. Discussion

In this thesis, key bacterial components of a belowground food web were identified by labelling experiments in the lab and then located back in their natural habitat (an agricultural field) with regard to their spatio-temporal abundance and distribution. Bacterial degraders of specific plant-derived substrates in soils have already been identified in previous studies (e.g. Dilly *et al.* 2004, Haichar *et al.* 2012, Padmanabhan *et al.* 2003) but rarely with an explicit food web perspectives. Bacteria are only depicted as 'black box' in current soil food web models which is contrary to their high functional diversity in soils. In this thesis, I did not only identify bacterial consumers of different substrates with varying complexity and recalcitrance from the same soil, but I also analysed their specific distribution and abundance patterns in an agricultural field with regards to soil compartments and seasonality. Further, I aimed to assess the specific contribution of identified key populations to carbon fluxes in the soil food web. With this study I want to contribute to the endeavour for improved implementation of bacterial taxonomic and functional diversity in soil food web concepts (Allison *et al.* 2008, McGuire *et al.* 2010, Paterson *et al.* 2009) with actual field data. This is enabled by a concerted and interdisciplinary field study from the DFG Research Unit FOR 918, where food web members of all trophic levels (micro-, meso- and macrofauna) were identified from the same soil together with assessment of carbon flux quantities.

4.1. Field sampling and investigation of the bacterial community distribution *in situ*

To verify the general suitability of the chosen exploratory field site in Holtensen for this approach, and to obtain a baseline for assessment of field distribution patterns of the identified key food web members, the overall bacterial field microbiota was investigated in an extensive sampling campaign. The field was sampled in May, July, September and December 2009 to cover all variations in season and plant cultivation states parameters (seeding, flowering, fructification and decay). An additional sampling took place in July 2010 to compare seasonal effects of maize growth on the bacterial community composition. Besides seasonal influence on the bacterial community, soil compartment (bulk soil, rhizosphere and root surface) was considered, as well as soil depth (top soil, plough layer, subsoil). Different cultivation treatments of the sampling plots carried out by the Research Unit accounted for distinct substrate specific carbon inputs to the soil: Plots were cultivated with either maize or wheat, and to every second of both cultivation types maize shoot litter was added after harvest.

4.1.1. Spatial heterogeneity

Prior to field plot installation, a vertical and a horizontal transect were sampled in the field to assess the spatial heterogeneity of intrinsic microbiota, as well as the comparability of plots before they were cultivated with distinct treatments. Only very little changes were observed in the bacterial community composition in 6 triplicate samples along a 125 m horizontal transect of the top soil. In contrast, high variation was found over the depth transect from top to subsoil (65 cm). Horizontal homogeneity derived most likely from the constant agricultural land use of the field over decades and horizontally consistent soil parameters. Generally, an absence of pronounced variation of microbial communities in physico-chemically homogeneous habitats is common (Musslewhite *et al.* 2003, Robinson *et al.* 2009). This horizontal homogeneity of bacterial communities was a key prerequisite to install the different cultivation treatments, as it ensured that observed community shifts would relate to treatment effects and were not already inherent in the soil. Furthermore, this homogeneity enabled us to use composite samples from 3 plots with the same cultivation treatments as biological triplicates in our experiments. MANOVA analysis of the T-RFLP fingerprinting data from the actual sampling campaign further verified that no significant influence on the overall variance was caused by plot origin of the sample. In the actual sampling campaign small scaled spatial heterogeneities were further mitigated as the respective depth segments from 8 to 10 sampling cores were pooled from each plot to gain enough soil material to provide each member of the Research Unit with the same soil sample. This ensures comparability of the findings for all different organisms of the soil food web investigated in the Research Unit and was especially important to unambiguously identify putative treatment effects in the field experiment. Sample pooling can affect estimations of the microbial diversity (Manter *et al.* 2010). Here, however, the benefits of pooling prevailed over such disadvantages, particularly as the focus of this thesis was on specific bacterial key food web members and less about rare taxa and overall community diversity.

4.1.2. Bacterial distribution between different soil compartments

The distribution and abundance of soil bacteria was investigated with regard to soil compartment, soil depth, sampling time and cultivation treatment. Especially soil compartment (bulk soil, rhizosphere and root surface) had crucial impact on the bacterial field community composition and mostly the root surface, but also the rhizosphere

harboured a widely different community from bulk soil. A selective enrichment of certain bacteria from the adjacent soil and altered overall communities structures have been often reported for rhizosphere (Berg *et al.* 2009, Hartmann *et al.* 2009a, Mougel *et al.* 2006). As whole roots were used to extract DNA and RNA from root surface, a small amount could in fact origin from bacteria within the roots. However, the vast majority of extracted nucleotides should be from the root surface as root fragments were still intact after nucleotide extraction.

On the maize root surface and in rhizosphere samples from the field, putative nitrogen fixing bacteria were highly abundant (*Rhizobium* spp., *Mesorhizobium* spp., *Bradyrhizobium* spp.), but also other bacteria like *Massilia* spp., *Mucilaginibacter* spp., *Pseudomonas* spp., and *Streptomyces* spp. that are assumed plant growth promoting rhizobacteria (Babalola 2010, Jankiewicz *et al.* 2012, Madhaiyan *et al.* 2010, Ofek *et al.* 2012). Plants select actively for such bacteria with plant growth promoting abilities like nitrogen fixation, phosphorous or sulphur supply, and synthesis of plant growth hormone analogues (Hayat *et al.* 2010). Such plant growth promoting rhizobacteria (PGPR) are often enriched in the rhizosphere. Other frequent root surface bacteria (*e.g. Dokdonella* spp., *Duganella* spp.) are known rhizoshere inhabitants albeit with less defined functional traits, (Haichar *et al.* 2008, Madhaiyan *et al.* 2013). Furthermore, *Niastella* spp., *Opitutus* spp. and *Ohtaekwangia* spp., three genera not described as enriched in the rhizosphere so far, were quite abundant here. However, many lineages were abundant both in bulk soil and rhizosphere like *Bacillus* spp., *Arthrobacter* spp. and bacteria affiliated to *Acidobacteria* subgroup 6.

The root surface and the rhizosphere are known ‘hot spots’ of bacterial activity in soils (Berg *et al.* 2005). Plenty of organic compounds are available here from root exudates, root tip mucilage and lysates from herbivorous feeding damage released into the soil (Dennis *et al.* 2010). Indeed, here, in contrast to bulk soil samples, 16S rRNA gene and 16S rRNA fingerprints from maize root surface samples were almost identical, indicating a high overall bacterial activity and very few dormant or dead cells in this habitat. Living bacterial cells harbour the same amount of 16S rRNA genes regardless whether they are active or dormant. In contrast, rRNA is much more abundant in active cells. Therefore, comparison of T-RFLP patterns from 16S rRNA and rRNA gene fingerprints for the same community has the potential to indicate the more active populations (Noll *et al.* 2005). 16S rRNA gene and 16S rRNA fingerprints of rhizosphere samples were more dissimilar: rhizosphere rRNA gene samples were still quite similar to the root surface fingerprints, but

rRNA fingerprints comprised several unique T-RFs. Part of these present (16S rRNA gene) but less active (rRNA) rhizosphere bacteria may have initially benefitted from root exudates while close to the tip of growing root ends, but were then no longer able to maintain this high activity as they experienced lower substrate supply on maturing roots. Also, root influence and availability of rhizodeposits decreased with a steep gradient with increasing distance from roots (Dennis *et al.* 2010). Already at 2 mm distance from a root, only one third of initial root exudate amounts can be detected (Kuzyakov *et al.* 2003).

Besides soil compartment, the bacterial community from the field was strongly influenced by soil depth. Top soil and the root free zone comprised a clearly distinguishable community composition. The middle layer around the plough sole, however, appeared to harbour a transitional community composition, most sensitive to soil water content (see discussion further down). Depth dependent shifts of soil microbiota have been reported frequently (Blume *et al.* 2002, Eilers *et al.* 2012, Hansel *et al.* 2008) and are independent of soil type (Fierer *et al.* 2003, Gelsomino *et al.* 2011), type of vegetation (Hartmann *et al.* 2009b) and land use (Will *et al.* 2010). The most eminent soil properties causing such depth effects were reported to be carbon and nitrogen content, pH, soil water content and electrical conductivity (Ganzert *et al.* 2011, Gelsomino *et al.* 2011, Will *et al.* 2010). At the exploratory field, impact of depth on the bacterial community correlated with total organic carbon (TOC), extractable organic carbon (EOC) and total nitrogen (Kramer *et al.* 2012, Scheunemann *et al.* 2010). Coherence with pH is also very plausible, but pH was not consistently measured for all sampling time points. Soil moisture, however, appeared not essential for depth effects on soil bacteria at our exploratory field although a decrease of soil water content with depth was observed. Bacterial abundances decreased markedly with depth and the subsoil hosted only about one-tenth of the bacterial populations found in the top soil. Reduced bacterial biomass in deeper soil layers is frequently observed (Berg *et al.* 2007, Eilers *et al.* 2012, Griffiths *et al.* 2003), together with a generally lower activity of the bacteria present in the depth (Griffiths *et al.* 2003). In my work, comparison of 16S rRNA gene and 16S rRNA fingerprints also supported a higher metabolic activity of the top soil bacteria compared to those in the root free zone throughout the year.

Some of the more abundant taxa showed clear preference of top soil (*e.g. Nocardioides* spp., *Marmoricola* spp.) or the subsoil (*e.g. Nitrospira* spp., *Geobacter* spp.). On a phylogenetically broader scale, abundances of *Bacteroidetes*, *Alpha-* and *Gammaproteobacteria* tended to decrease with depth at the exploratory field. To some extent, read abundances of *Actinobacteria* appeared to be reduced with depth, too.

However, relative sequence abundances of *Actinobacteria* appeared not very robust between different sequencing runs as will be discussed below. Depth effects on those phyla have also been observed by others (Eilers *et al.* 2012, Will *et al.* 2010) together with a decrease of *Betaproteobacteria* and an increase of *Acidobacteria*, which was not confirmed by my findings. Thus, the overall bacterial community in the field displayed strong effects in regard to soil compartment (bulk soil, rhizosphere, root surface) and depth on community composition, abundance and activity. This already suggests that at least some bacterial taxa exhibit a distinct specialisation for certain soil compartments and coincides with my second initial hypothesis that bacterial food web members are subject to spatio-temporal dynamics in the field.

4.1.3. Seasonality and treatments

Seasonal changes and cultivation treatment had small but significant influence on the bacterial community in the field. Statistical analyses correlated seasonal influence mostly with soil water content. Indeed, the bacterial community from the plough sole was more similar to that from the subsoil for the sampling time points with lower soil water content in September and July 2010. In the month with higher soil water content, plough layer bacteria resembled more the top soil community. For top soil and subsoil bacteria, however, seasonal effects were not related to soil water content. Here, summer samples (July 2009 (wet), July 2010 (dry)) harboured different communities in comparison to all other time points. Therefore, other parameters than soil water content must have caused this. Temperature could be an influencing parameter as it was observed to be important for seasonal changes of bacterial communities (Blume *et al.* 2002, Braker *et al.* 2010, Pettersson *et al.* 2003).

No clear seasonality was observed for maize rhizosphere and root surface samples. This was unexpected as rhizodeposits change with plant growth stage (Hartmann *et al.* 2009a) and clear seasonal effects on the bacterial rhizosphere community have been reported previously (Gomes *et al.* 2001, Houlden *et al.* 2008). For rhizosphere and root surface communities, samples from December 2009 and July 2010 differed from the earlier samples. This could neither be explained by direct plant influence nor any other measured parameter. The field had not been cultivated with maize plants for decades and therefore a slow adaptation of intrinsic microbiota to the maize rhizosphere might have induced the shift. Only in July 2010, after one year of maize cultivation, the bacterial community from wheat and maize soil samples differed clearly (Scharroba *et al.* 2012). This could support

the adaptation theory but this can only be unambiguously proven by continued sampling campaigns on the exploratory field. Already Smalla et al. (2001) have observed a stronger seasonal rhizosphere effect for the second year of cultivation with new plant species (Smalla *et al.* 2001). Litter may also have contributed to this shift of the rhizosphere community composition in December, as rhizosphere and root surface samples were taken from the corn maize plots, where maize litter was added only after the September 2009 sampling and harvest. However, rhizodeposits and dead root materials should be the main carbon source for the rhizosphere microbiome, and litter amendment should not significantly affect root surface or rhizosphere bacteria. Even more so, since a clear litter effect was not even observed for bulk soil samples, where it was most expected.

Changes of soil microbial communities after plant litter amendment have mostly been reported for litter bag experiments (Bray *et al.* 2012, Dilly *et al.* 2004, Marschner *et al.* 2011). Litter, and especially its polymeric compounds, can only be degraded with extracellular enzymes (Gilbert *et al.* 2008) and is therefore most effective in direct proximity of the microbes to litter particles. Thus, degrading bacteria can be quite abundant in litter bags, but still quite rare in the overall bulk soil as sampled in our field work and therefore, no strong litter effect was observed.

Taken together, depth and rhizosphere effects were identified as the most important drivers of overall bacterial community composition in the field. Seasonal and plant cultivation influences were less pronounced, but typical for agricultural soils in temperate climates. The chosen exploratory field thus represents an adequate site to localise specific bacterial populations relevant for carbon fluxes in soils and their spatio-temporal distribution patterns: First, the horizontal homogeneity of bulk soil bacterial communities within soil horizons facilitate a clear assignment of observed variations to experimental variables (depth, soil compartment, time, cultivation treatment). Second, the findings derived from this thesis can be generalised, since comparable effects of determined experimental environmental variables on soil bacterial communities have also been observed elsewhere.

4.1.4. General methodological considerations regarding data validity

In this study, relative 16S rRNA gene abundances were assessed via amplicon pyrosequencing and considered as relevant information on taxon abundances in soil. Since pyrosequencing is a relatively new method, its semi-quantitative accuracy is still discussed. Although some reports are quite supportive (Lee *et al.* 2012, Pilloni *et al.* 2012a), it is clear that quantitative assertions inferred from such data sets must be interpreted with caution

(Amend *et al.* 2010). Especially, if samples are not analysed in biological replicates due to the still considerable sequencing costs and the time required for data analyses.

Therefore, in parallel to pyrotag sequencing, I also used T-RFLP fingerprinting of soil DNA extracts, with three biological replicates (soil and root samples, results part 2, 3 and 4) and technical replicates (lysimeter water samples, results part 4) to verify the most important findings from amplicon sequencing. T-RFLP fingerprinting is known to allow for a robust semi-quantitative evaluation of OTU abundances between samples treated in the same way (Schütte *et al.* 2008). The approach to combine T-RFLP fingerprint with pyrosequencing data was already applied successfully previously and allowed for reliable semi-quantitative assumptions based on relative sequence abundances (Pilloni *et al.* 2012a, Shani *et al.* 2013, Weissbrodt *et al.* 2012, Ziganshin *et al.* 2013), therefore this strategy can be regarded with confidence.

In results part 3 and 4, sequence abundances are not only used for semi-quantitative comparison, but also for the quantitative estimation of cell numbers and respective biomass carbon in combination with qPCR results. Clearly, those numbers should be regarded as cautious estimates. Comparing read abundances of a specific taxon from equally treated samples should adequately resemble actual abundance fluctuation, as sequencing biases should be similar (Amend *et al.* 2010). Quantitative interpretation of these data can be biased by sequencing errors, the use of distinct adapter-elongated primers, different amplification efficiencies and all other common PCR biases, which can even be multiplied here, as several PCR steps are involved in amplicon sequencing (Lee *et al.* 2012). Even more so if pyrotag abundance is combined qPCR, as introduced in this thesis.

Still, I am convinced that these cell counts are relevant at least to certain extends, supported also by a general congruency of fingerprinting and sequencing data sets in this study: All abundant reads of bacterial taxa labelled in the SIP experiments could be assigned to a T-RF enriched in ^{13}C. Also, bacterial taxa, highly abundant only in rhizosphere and root surface, could be assigned to a T-RF important for rhizosphere/root surface samples according to PCA. Furthermore, the PCA patterns of soil and lysimeter samples were almost identical for T-RFLP fingerprinting and pyrosequencing data for the mobilisation and transport experiment in results part 4. Most reassuring for the validity of my cell count estimations based on pyrotag read abundances and qPCR was the fact, that the magnitude of microbial carbon in the soil samples of part 4 (bacterial transport by seepage water) was comparable to my estimations. Microbial carbon from the field

samples was measured by chloroform-fumigation-extraction by Susanne Kramer (University of Hohenheim)

Unfortunately, a taxon-specific systematic error may have occurred in pyrosequencing of the field sampling campaign as pyrosequencing was not done in a single run as for the other experiments. Rather, sequencing of field samples spread over two years and inconsistencies were observed especially regarding the abundances of *Actinobacteria*. Here, overall read abundances varied between ~15 and 25% depending on the sequencing date. PCR biases with universal primers for *Actinobacteria* have been reported before (Farris *et al.* 2007) and could probably be connected with observed GC biases in PCR and pyrosequencing (Benjamini *et al.* 2012) as not only *Actinobacteria* genomes are GC rich but also their 16S rRNA genes present in the LTP 111 data base of the ARB-SILVA-project (Munoz *et al.* 2011) of all cultivated *Actinobacteria* show slightly higher GC content (58.3 %) in comparison to all other cultivated bacteria (54.1 %) from this data base. As all sequencing runs and data analysis was done via identical procedures, changes in 454 chemistry could be assumed to be responsible. A distinct drop of overall *Actinobacteria* relative sequence abundance occurred for runs from February 2011 and a change for Short Fragment Removal Procedure at this time was published by the manufacturer (Roche Diagnostics, 2011). However, not all *Actinobacteria* taxa appeared affected to the same extend and most differences were observed for unclassified *Actinobacteria*, unclassified *Actinomycetales* and *Nocardioidaceae*. Taxa identified to be relevant in carbon flow (*Artrobacter* spp, *Humicoccus* spp., *Kitasatospora* and unclassified *Streptomycetaceae*) were less affected by this bias. Therefore, those 16S rRNA gene abundances were used in quantitative estimations, as well.

Finally, ^{13}C-labelling of taxa in SIP gradients was assessed by carefully comparing respective T-RF abundances, as it is commonly done (Pilloni *et al.* 2012a, Shani *et al.* 2013, Weissbrodt *et al.* 2012, Ziganshin *et al.* 2013) A particular T-RF was defined as labelled only when it was distinctly more abundant in the 'heavy' ^{13}C fractions in comparison with both 'light' ^{13}C fractions and all fractions of the ^{12}C control. However, in addition, I also introduce the use of comparative pyrotag enrichment within 'heavy' fractions to infer labelling. Pyrosequencing was done for one 'heavy' and one 'light' fraction for ^{13}C and respective ^{12}C gradients only, thus no step-wise information from 'heavy' to 'light' fractions was available. Here, the metre for enrichment was an indicator that included the respective read abundances from all sequenced 'heavy' and 'light' fractions from both ^{12}C and ^{13}C treatment. This is an approach analogous to the 'T-RF

subtraction values' introduced recently for SIP by Zumsteg *et al.* (2013), but template abundance in ^{12}C control fractions were also considered. Together with total read abundance in the 'heavy' ^{13}C rRNA fraction, these enrichment indicators are a promising tool to assess the involvement of pyrotag-defined taxa in specific substrate utilisation. Of course, they also have to be considered within the general limitations of PCR and sequencing biases.

Thus in general, although my findings of (semi-)quantitative pyrotag abundances should still be considered with caution, this approach has the great advantage, that assumptions on the involvement of specific taxa in carbon flow now become methodologically feasible for many, also low-abundance taxa all in one approach. Moreover, pyrotag read abundances have been reported to be robust and meaningful (Pilloni *et al.* 2012a) and I could confirm the validity of all respective interpretations for major bacterial taxa involved in carbon flow by comparison with results from other, well established methods like T-RFLP fingerprinting and microbial carbon measurements.

4.2. Identification of key bacterial food wed members by stable isotope probing

Within the Research Unit, three ^{13}C RNA SIP experiments were conducted to identify microbial utilisers of various substrates with different complexity and recalcitrance. Besides help with planning and accomplishing of the rhizosphere and detritussphere substrate SIP experiments, I planned and conducted the microcosm experiment with labelled bacterial biomass. Furthermore, I performed SIP for all those experiments and analysed bacterial rRNA from the SIP gradients with T-RFLP fingerprinting and amplicon sequencing.

With these experiments we identified key bacterial food web participants degrading rhizodeposits, maize root and leaf litter (and the model substrates glucose and cellulose). Furthermore, some inter-bacterial secondary trophic links were identified as well. The same soil from the explorative field was used in all microcosm experiments. Other Research Unit members investigated labelled fungi (Buscot lab, UFZ Magdeburg) and protozoa (Bonkowski lab, Univ. Köln) to allow for comprehensive insights into the microorganisms and their trophic interaction of this soil food web.

4.2.1. Bacterial rhizodeposit utilisers

Maize plants grown in rhizoboxes were kept under a $^{13}CO_2$ atmosphere by daytime for 6 days to identify microorganisms utilising rhizodeposits as carbon source. RNA from the

rhizobox soil samples was taken at intervals over 16 days and bacterial RNA was investigated with SIP and subsequent 16S rRNA T-RFLP fingerprinting for all gradient fractions. Based on the fingerprinting results, defined fractions were selected for amplicon pyrosequencing.

Although it has been reported that rhizodeposits can be taken up and incorporated into RNA from rhizosphere microorganisms within several hours (Vandenkoornhuyse *et al.* 2007), no labelled T-RFs were found in our experiment after 1 and 3 days. As unlabelled rRNA tends to occur also in 'heavy' fractions and over entire CsTFA gradients, only T-RFs distinctly more abundant in the 'heavy' ^{13}C RNA fractions than in all other ^{13}C and ^{12}C fractions are considered as labelled (Lueders *et al.* 2004). In contrast, Vandenkoornhuyse et al. (2007) defined all sequences found in the heavy fractions as labelled, although many identical sequences were also found in the light fractions. They argued that those bacteria used both fresh ^{13}C labelled and also old unlabelled substrates as carbon source. To prevent false positive interpretation, a more rigorous definition of 'labelled' is used in this thesis.

Nevertheless, if only a small subgroup of a given soil population actually used ^{13}C-labelled rhizodeposits due to favourable localisation near the roots, they would not be identified as labelled according to my definition.

After 5 and 8 days of the experiment, only a small number of T-RFs appeared labelled. As the rhizoboxes were practically full of roots, all soil was considered as rhizosphere initially. However, to be on the safe side, 'classical' rhizosphere samples were also obtained and analysed in SIP. Here roots were shaken and remaining adherent soil was defined as strict rhizosphere (Buddrus-Schiemann *et al.* 2010). However, from these samples, only few labelled T-RFs were identified as well. The identified labelled T-RFs and bacterial taxa differed for rhizobox soil and rhizosphere samples. Generally, more and earlier labelled OTUs were observed for the rhizobox soil. As the soil in the rhizoboxes was very dense and tightly adherent to the roots, it is possible that fine roots were severed by the attempt to shake of bulk soil compartments. This would mean that in this case the two sampling methods rather resembled rhizosphere soil from fine and mature roots. As root exudates are excreted by fine roots, this would explain the unexpected findings that more ^{13}C was assimilated in the overall soil of the rhizoboxes than in the supposed rhizosphere soil.

Amplicon pyrosequencing of one 'heavy' and one 'light' RNA fraction each of the ^{13}C and ^{12}C SIP gradients revealed, that labelled T-RFs mostly belonged to *Opitutus* spp.

(*Verrucomicrobia*). Only one species of this genus is known so far: *O. terrae*, an obligate anaerobe isolated from a rice paddy soil (Chin *et al.* 2001). The three known strains from this species possess a fermentative metabolism with propionate and acetate as the major end products (Schlesner *et al.* 2006). Generally, *Verrucomicrobia* are abundant and possibly important bacteria in the rhizosphere but they are largely ignored by researchers so far (Kielak *et al.* 2010). So far, no other study has revealed *Opitutus* spp. as an important rhizodeposit utiliser, but they appear to be key organisms at the exploratory field, as pyrosequencing reads affiliated to the genus were generally quite abundant at root surface.

Amplicon pyrosequencing and comparison of read abundances revealed a much more diverse population of bacterial rhizodeposit utilisers than the T-RFs. Highest ^{13}C enrichment was found for *Mucilaginibacter* spp., *Opitutus* spp., *Sphingobium* spp., *Ohtaekwangia* spp. and *Massilia* spp.. Exept *Opitutus* spp., none of them had high relative sequence abundances despite their high ^{13}C incorporation. This discrepancy explains their failed detection in T-RFLP fingerprinting, as any T-RF of a given length can comprise several taxa (Weissbrodt *et al.* 2012). Species affiliated to *Sphingobium* and *Massilia* are both often root associated and can be very abundant in the rhizosphere of maize (Balkwill *et al.* 2006, Dohrmann *et al.* 2013). In addition, *Massilia* spp. are putative plant growth promoting rhizosphere bacteria (PGPR) and partly even root inhabiting (Ofek *et al.* 2012). They were also very abundant in the rhizosphere and at the root surface from the exploratory field. Bacteria affiliated to the genus *Mucilaginibacter* are also often found in rhizosphere (Lee *et al.* 2013) and at least two species are known as PGPR (Madhaiyan *et al.* 2010). They are non-motile and produce a lot of extracellular polymeric substances (EPS) (Pankratov *et al.* 2007). So far, only two species of *Ohtaekwangia* are known, a deep-branching genus of *Bacteroidetes*. *O. koreensis* and *O. kribbensis* are both non-motile and aerobic bacteria, isolated from marine sediment (Yoon *et al.* 2011). Nevertheless, a very close relative, *Chryseolinea serpens*, was isolated from soil (Kim *et al.* 2013) and bacteria affiliated to *Ohtaekwangia* were also recently found in the rhizosphere of sunflowers (Tejeda-Agredano *et al.* 2013).

Read abundances affiliated to many other bacteria suggested ^{13}C enrichment of a very diverse population. However, their enrichment with ^{13}C and their relative abundances in the 'heavy' ^{13}C fraction were low and therefore probably less relevant for the soil food web, as only little rhizosphere carbon was incorporated by them. Others, like *Arthrobacter* spp., *Singulisphaera* spp., *Azospirillum* spp. and unclassified *Planctomycetaceae* were

quite abundant in the 'heavy' ^{13}C fraction but had only little ^{13}C enrichment as they were also abundant in all other SIP gradient fractions. Those could be important in using rhizodeposits as 'activation energy' to oxidize other pools of SOM (the so-called 'priming effect') (Kuzyakov 2010). Also, spatial heterogeneity of substrate distribution could have provided only some of the bacteria affiliated to *Arthrobacter* spp. with rhizodeposits and therefore with ^{13}C carbon. Both *Azospirillum* spp. and *Arthrobacter* spp. possess PGPR properties (Babalola 2010). Under grass turfs, mostly *Burkholderia* spp., but also unclassified *Betaproteobacteria* and *Sphingomonadaceae* were utilising rhizodeposits (Vandenkoornhuyse *et al.* 2007). Besides general rhizosphere inhabitants like *Actinomycetales* and *Sphingobacteriales*, maize rhizodeposits have been previously reported to be utilised by *Azospirillum* spp., *Sphingomonas* spp. and *Dokdonella* spp. (Haichar *et al.* 2008).

Generally, such SIP experiments can only show a snapshot of rhizodeposits-dependent carbon flow, and only the most prominent rhizodeposit utilisers can be identified here. The rhizosphere is a very dynamic habitat (Buée *et al.* 2009) and root growth constantly causes relocation of the carbon source. Therefore, population sizes of both copiotrophic and oligotrophic bacteria oscillate wavelike along roots due to growth and death cycles (Semenov *et al.* 1999). Although probably not all rhizodeposit utilising bacteria were detected with this experiment, some key bacterial food web members were indeed identified. Some of them are known as plant growth promoting rhizosphere bacteria (*Azospirillum spp., Arthrobacter spp., Mucilaginibacter* spp, and *Massilia* spp.), others are well-known rhizosphere inhabitants (i.e. *Sphingobium* spp., *Rhizobacter* spp. and *Kitasatospora* spp.). Others, however, were so far not known to be abundant or relevant in the rhizosphere (*Opitutus* spp. and *Ohtaekwangia* spp.).

The findings of this experiment demonstrate that rhizodeposits are mainly converted by specific subpopulations of rhizosphere bacteria at a given time. With regard to my initial hypotheses about the bacterial 'black box', this clearly indicates that it is not to sufficient to consider overall bacterial biomass as an adequate foundation for the modelling of carbon fluxes in soil food webs. Rather, the specific sub-populations utilising defined substrates should be discerned.

4.2.2. Key detritusphere bacteria

Microcosm experiments with ^{13}C-labelled maize leaf and root litter and also the two model substrates glucose and cellulose were conducted to identify litter degraders and their

response to substrates of varying recalcitrance and complexity. Glucose can in fact be utilised by a vast diversity of microbes, whereas cellulose can only be degraded by bacteria with cellulolytic exoenzymes (Berlemont *et al.* 2013). Leaf and root litter are complex substrates mostly consisting of (partly crystalline) cellulose, hemicellulose and lignin. Root litter comprises more recalcitrant compounds and is even less degradable than leaf litter (Fujii *et al.* 2010).

In this experiment, glucose was predominantly degraded by *Arthrobacter* spp. and also by other unclassified *Micrococcaceae*, *Pseudomonas* spp. and later also *Humicoccus* spp.. Bacteria affiliated to *Arthrobacter* spp. are very common in soils and can use a huge variety of substrates as carbon source including polymeric compounds and herbicides (Westerberg *et al.* 2000). Apparently, they are also very competitive glucose utilisers (Padmanabhan *et al.* 2003, Schellenberger *et al.* 2010). As in the rhizodeposit experiment, *Arthrobacter* spp. reads were very abundant in the ^{13}C 'heavy' fraction, but their specific enrichment in 'heavy' RNA was not as pronounced as for other, less abundant templates. Potentially, *Arthrobacter* spp. can effectively use the priming effect of added glucose to utilise other more recalcitrant SOM. Bacteria affiliated to the *Actinobacteria* have been frequently associated with priming effects (Bastian *et al.* 2009, Bernard *et al.* 2007). Later glucose degradation also involved *Humicoccus* spp. And the deferred labelling could indicate better adaptation for low concentrations of soluble organics.

Carbon assimilation from cellulose was dominated by *Cellvibrio* spp. and *Flavobacterium* spp. at the early time points and by bacteria affiliated to *Kitasatospora* spp. and other unclassified *Streptomycetaceae* after 32 days. Polymeric cellulose can only be degraded by extracellular enzymes and only the cleavage products, mainly glucose and cellubiose, can be assimilated. Therefore, 'cheater' bacteria without cellulolytic abilities that utilise soluble cellulose derivates liberated by others can also assimilate cellulose carbon. And indeed, *Flavobacterium* spp., was highly ^{13}C enriched at an early degradation stage, and is not known to degrade crystalline cellulose but only soluble derivatives (Bernardet *et al.* 1996). Cellulolytic activity of the later degrader *Kitasatospora* spp. is also debatable, as none of the validly described species are known to degrade cellulose so far (Kämpfer 2006), although it was reported for an isolate from soil affiliated to *Kitasatospora* (Ulrich *et al.* 2008). In contrast, *Cellvibrio* spp. (labelled early, Lynd *et al.* 2002) as well as many strains of the *Streptomycetaceae* (labelled late, Kämpfer 2006) are well-known cellulose degraders. Besides differentiation because of substrate concentrations, top-down controls could also have caused this marked succession of cellulose degraders. Both bacterial and

eukaryotic micro-predators favour to prey on Gram-negative bacteria (*Flavobacterium* spp., *Cellvibrio* spp.) over Gram-positive bacteria (*Streptomycetaceae*), and could have driven this switch in key cellulose degraders.

Unexpectedly, both leaf and root litter were utilised by the same bacterial taxa and the higher recalcitrance of root litter did not result in different key consumers. However, rRNA enrichment indicators and abundances in the 'heavy' ^{13}C fractions varied. Early litter carbon assimilation was observed mainly for *Flavobacterium* spp., *Cellvibrio* spp., C*ytophaga* spp. and *Mucilaginibacter* spp. Later, *Ohtaekwangia* spp. and several *Polyangiaceae* (*Byssovorax* spp., *Sorangium* spp. and unclassified species) also incorporated litter carbon. Litter comprises many substrates for bacteria such as cellulose, hemicellulose as well as less recalcitrant substrates like pectin, xylan and starch. Especially *Flavobacterium* spp., *Mucilaginibacter* spp. and *Ohtaekwangia* possibly utilised those less recalcitrant compounds as they were only observed at early degradation and no cellulolytic activity has been reported for theses lineages (Bernardet *et al.* 2002, Pankratov *et al.* 2007, Yoon *et al.* 2011). In fact, *Flavobacterium* spp. was labelled with all substrates and was always more abundant at early degradation. This may indicate a copiothropic lifestyle and preference for easily degradable substances.

Polyangiaceae are myxobacteria, gliding bacteria with complex life cycles and various extracellular enzymes for degradation of macromolecules and biopolymers. Additionally, they can also act as micropredators (Reichenbach 1999). Although they are also cellulolytic and are specialised as degraders of complex organic substrates, an involvement as secondary predators of primary litter degraders is very plausible, as they are only labelled after 32 days.

All in all, bacterial detritussphere populations varied with respect to the different substrates and degrader diversity increased with elevated complexity. This was also evident from indices of functional organisation analysis (*Fo*, Marzorati *et al.* 2008), which was generally higher for the 'heavy' RNA of litter degraders in comparison to glucose and cellulose degraders. Glucose and cellulose were mainly degraded by one or two dominant bacterial taxa. In contrast leaf and root litter were utilised simultaneously by several taxa with lower T-RF and read abundances in heavy rRNA and without clear dominances. Glucose is a universal substrate and therefore competition is high for this compound. Apparently, the bacteria with the fastest consumption rate and highest proliferation rates won the race and became the dominant degraders. Cellulose is more recalcitrant and only bacteria with extracellular enzymes can attack the polymers. Still, the extracellular break-down of

cellulose provides glucose and cellobiose also for 'cheater' bacteria not able to degrade cellulose themselves (Štursová *et al.* 2012). Sometimes bacteria can even cooperate for more effective cellulose degradation (Kato *et al.* 2004). Therefore, several pathways for carbon assimilation from cellulose coexist, and this can already generate a higher diversity of involved degraders than for glucose alone, although cellulose is also a single compound substrate. Highest degrader diversity was observed in the microcosms with litter as and no dominating consumer taxa were identified, here. Generally, more complex substrates like litter are hypothesised to feed more diverse bacterial population (Dungait *et al.* 2013) as more substrate compounds offer more feeding niches and more specialists are needed here for degradation (Dilly *et al.* 2004). Also, plant residues contain many biopolymers like cellulose, starch, pectin and lignin. As glucose is also produced as an intermediate product of cellulose degradation, overlapping population were expected here. The lack of overlap may result from different feeding strategies of respective degrader populations, and challenges the relevance of glucose as a model substrate in soils.

For all substrates a more or less pronounced shift in degrader community composition was observed over time. This is often observed for litter degradation ((Bray *et al.* 2012, Dilly *et al.* 2004) and is mainly attributed to two aspects: bacterial 'lifestyle' and increasing litter recalcitrance. But also selective grazing of predatory microbes could induce such successions as mentioned above. The shifting community of glucose degraders was probably mainly attributed to different bacterial lifestyles. Fast growing copiotrophs can optimally deal with high substrate concentrations and respond readily to easily degradable substances. They become less competitive when substrate supplies decrease which favours oligothrophs adapted to low substrate concentrations and with slower proliferation rates (Fierer *et al.* 2007). A similar effect could be true for cellulose degradation but cellulose is generally degraded slower as exoenzymes are not constantly prepared but have to be produced anew whenever the substrate is present which causes a time lag of degradation. For plant litter, substrate composition of litter changes during degradation and easily degradable soluble substances like glucose are depleted within days, whereas more recalcitrant substances like lignin can persist for several months (Yadav *et al.* 2007). In my results, both bacterial 'lifestyle' and progressing litter degradation may have caused degrader succession. Copiotrophic bacteria likely first degraded most of the soluble substances, and oligotrophs became increasingly competitive over time. Comparing ^{13}C enrichment and read abundances in the 'heavy' ^{13}C fraction from our results, it can be

suggested that *Flavobacteria* spp, *Mucilaginibacter* spp, *Cellvibrio* spp. and *Cytophaga* spp. are rather copiothrophic.

Carbon from all substrates was obviously assimilated by bacteria, regardless their varying complexity and recalcitrance. All substrates induced different consumer populations, and clear substrate specialisations can be supposed. As hypothesised, this contradicts the routine perception of soil bacterial biomass as one 'black box', assumed to be involved chiefly in the degradation of labile organic matter (Moore *et al.* 2003). Rather, key bacterial degraders are specific and specialised taxa, and distinct population are not only involved in degradation of easily but also more recalcitrant substrates.

4.2.3. Bacterial biomass amendment

In this SIP experiment, my aim was to identify secondary intra-microbial trophic links. ^{13}C labelled bacterial biomass was added to the microcosm soil. Added bacteria were initially enriched from the field soil with naturally concentrated ^{13}C model substrates (and ^{12}C as parallel control) often reported to be found in rhizoexudates (Marx *et al.* 2010).

These highly ^{13}C labelled putative root exudate utilisers were added to the microcosms and soil bacteria and microeukaryotes feeding on these added bacteria (as predators or saprophytes) were expected to incorporate labelled carbon into their own biomass. Preliminary tests had revealed that overall diversity in the model exudate enrichment cultures decreased with every transfer to fresh medium. Therefore, bacteria of the enrichment culture were added to the soil without prior identification of the enriched taxa. Three taxa were enriched and abundant in the amendment: *Pseudomonas* spp., *Cupriavidus* spp. and *Burkholderia* spp.. Remarkably, none of these were labelled in the original rhizodeposit SIP experiment. Still, I am confident that those added bacteria can be considered as an adequate model for exudate utilisers, as *Pseudomonas* spp. and *Burkholderia* spp. were both very abundant in the field rhizosphere and on the root surface in the field. Furthermore, all three taxa are PGPR and often found in rhizospheres (Hayat *et al.* 2010) and *Cupriavidus* spp. and *Burkholderia* spp. are known as symbiotic root nodule bacteria (Lee *et al.* 2006).

Throughout the experiment (12 days) bacterial 16S rRNA in the 'heavy' ^{13}C fractions predominately comprised the T-RFs of the added bacteria. Only few other T-RFs were observed after 1, 3 and 8 days of the amendment. After 12 days, only the three initially added taxa remained labelled. Apparently, the ^{13}C label was not effectively taken up by microbial secondary consumers, probably due to a marked persistence of the added bait

bacteria. A similar survival rate was observed for *Pseudomonas lurida* by Crotty *et al.* (2011) who tracked bacterial stable isotope label into soil invertebrates. In contrast, ^{13}C labelled *Escherichia coli* from another study was no longer detectable one week after amendment to a soil microcosm. Here, main secondary bacterial consumers were bacteria related to *Lysobacter* spp., the *Myxococcales* and within the *Bacteroidetes* (Lueders *et al.* 2006). Not only the *Myxococcales*, but also *Lysobacter* spp. possess micro-predatory abilities (Jurkevitch 2007) and also some members of the *Bacteroidetes* seem capable to thrive on other bacteria as substrate (Banning *et al.* 2010). The main reason, that presumed micro-predators were not labelled here could be the sieved microcosms soil. Generally, excretion of exoenzymes for the degradation of biopolymers and gliding motility for effective translocation in soil favour predatory abilities in bacteria (Nett *et al.* 2007). The soil used in the microcosms of this experiment was sieved and then stirred to ensure equal distribution of the suspension with enriched bacteria. Potentially, the disruption of the soil structure in this experiment could have inhibited putative bacterial micropredators in reaching their prey. This is even more plausible as the soil was not compacted to natural density for the substrate experiments microcosms. Potentially, the duration of the experiment was also too short, as myxobacteria were only labelled after 32 days in the detritussphere substrate experiments.

Instead, mostly two other bacterial taxa, *Diaphorobacter* and *Acinetobacter* spp., both not recognized as micropredators or saprophytes so far, were distinctly labelled 1 and 3 days after amendment. *Diaphorobacter* spp. are involved in nitrification and denitrification (Khardenavis *et al.* 2007) in soils and can degrade several aromatic compounds like 3-nitrotoluene (Singh *et al.* 2013). *Acinetobacter* spp. can also utilise a variety of compounds like glucose, phenol, caffeine and naphthalene (Padmanabhan *et al.* 2003) and also other aromatics (Thangaraj *et al.* 2008). The range of abilities is broad and the genus *Acinetobacter* comprises both phosphate solubilising PGPR (Babalola 2010) as well as nosocomial pathogens with high antibiotic resistances (Garcia-Quintanilla *et al.* 2013). Presumably, all identified secondarily labelled bacteria from this experiment utilised substrates from the bait amendment that were lysed during the process of biomass washing and stirring as after 12 days, 16S rRNA from the added bacteria themselves was still abundant in 'heavy' rRNA, in contrast to that of putative secondary consumers.

Therefore, this experiment largely failed to provide informative results on intra-bacterial predation of virtually natural soil bacteria. As mentioned above, others reported distinct predation of various bacterial taxa but the relevance and impact of this trophic interaction

on the overall carbon fluxes in the soil food web cannot be evaluated here. Should the experiment be repeated in the future, soil density should be adjusted to natural conditions and duration of the experiment should be prolonged to 32 days.

4.2.4. Synthesis of the SIP experiments

The microcosm SIP experiments revealed a large variety of actual plant-derived substrate utilising soil bacteria. Some are known as typical rhizosphere or detritusphere microbes, *e.g. Arthrobacter* spp., *Massilia* spp., *Flavobacterium* spp. and the *Streptomycetaceae*. Others were not observed to be important in plant-fed food webs so far, like *Ohtaekwangia* and *Diaphorobacter* spp.. Generally, all substrates were utilised by specific bacterial degrader populations depending on substrate complexity and recalcitrance. Higher substrate complexity (rhizodeposits and plant litter) was reflected in a higher diversity of involved degraders. In contrast, recalcitrance had only a minor influence on consumer diversity in our experiments, and was rather affecting degrader community composition, as a specialisation for these substrates is often required (*e.g.* exoenzymes, synthrophy). However, some bacteria appeared to have the ability to participate in the degradation of more than one substrates. Especially *Flavobacterium* spp. was utilising glucose but also assimilated carbon from litter and cellulose, potentially also from soluble compounds produced by other cellulolytic microbes. *Mucilaginibacter* spp. and *Ohtaekwangia* spp. were both enriched in ^{13}C from rhizodeposits and from litter, and *Kitasatospora* spp. assimilated cellulose and rhizodeposit carbon.

Putative priming effects were observed *e.g.* from *Arthrobacter* spp. and other *Actinobacteria*, indicating distinct bacterial involvement in the degradation of old and therefore more recalcitrant substrates. Unfortunately, proven bacterial micro-predators were not identified in the biomass labelling experiment, but instead, labelled myxobacteria were quite abundant at the later stages of litter degradation. Enrichment, however, was rather low and could therefore hint towards an involvement of cross-feeding. ^{13}C enrichment generally decreased with trophic interactions as mixed carbon sources find their way into consumer biomass. Myxobacteria were not labelled in the glucose SIP microcosms, which could contradict the predation hypothesis. On the other hand, many *Myxococcales* do not prefer Gram-positive prey bacteria like *Actinobacteria* (Morgan *et al.* 2010).

In essence, a variety of bacteria was using both easily available and recalcitrant substrates. Thus, the assignment of distinct ‘fast’ and ‘slow’ energy channels to the bacteria and fungi

(Moore *et al.* 2003) may be generally too simplistic. Even more so, as fungi were also observed to assimilate substantial amounts of carbon from all substrates in the detritussphere experiments, as reported by Julia Moll (UFZ, Halle) who analysed labelled fungal degraders in the same detritus SIP samples. Others also reported that fungi compete with bacteria for easy degradable compounds (Dungait *et al.* 2013) and anyhow, AMF are often the primary users of labile rhizodeposits (Drigo *et al.* 2010).

These findings indicate plausibly, that the assumption of strictly separated fungal and bacterial energy channels in current food web concepts is obsolete. Rather, as I initially hypothesised, all microbial organisms, be it bacteria or fungi, compete for all available substrates in soils regardless of substrate recalcitrance.

4.3. Back to the field: identified bacterial food web in their natural habitat

Most studies on substrate use of microorganisms in soil have either been conducted in SIP microcosm experiments, or they have described microbial communities found in substrate enriched soil. SIP microcosm experiments allow to specifically identify bacteria utilising defined substrates and incorporating respective stable isotope label. However, artificial microcosms may not always optimally reflect the natural conditions in the field, as soils are often disturbed and external parameters like *e.g.* weather are excluded (Bernard *et al.* 2007, Eichorst *et al.* 2012, Schellenberger *et al.* 2011, Vandenkoornhuyse *et al.* 2007). In contrast, mere community investigations of substrate added soils can reveal seasonal and spatial effects on putative degrader communities, but the microbes actually assimilating the substrate cannot be identified (Bjørnlund *et al.* 2006, Cavaglieri *et al.* 2009, Dilly *et al.* 2004, Eilers *et al.* 2010). Here, $^{13}CO_2$ pulse-labelling SIP studies directly conducted in the field have the potential to combine both approaches (Ostle *et al.* 2000, Rangel-Castro *et al.* 2005), but they are very challenging and expensive to conduct successfully, and may provide only a snapshot of the dynamic rhizosphere environment.

Therefore here, a unique and novel from-field-to-lab-to-field approach was chosen in this thesis. First, overall bacterial community composition in the field was investigated over different depths, seasons and treatments. Then, SIP microcosms were conducted to identify important bacterial food web participants for plant-derived carbon inputs to the soil. And finally – back to the field – the specific distribution patterns of these identified bacteria was assessed in their natural habitat.

This analysis of the distribution of the identified food web members in the field was based on 16S rRNA gene abundances determined by 454 pyrosequencing and qPCR. The

reliability of these data and their general consistency with T-RFLP fingerprinting results has been discussed above (4.1.4). 16S rRNA gene pyrosequencing data reflects percentages of specific taxa in relation to the overall community. As for any PCR based approach, conversion to cell numbers is not trivial and can be biased by varying 16S rRNA operon copy numbers in specific taxa. Since cell and biomass numbers are more relevant for food web modelling than gene counts, cell numbers were estimated from the relative sequence abundances together with taxon specific operon copy numbers and overall 16S rRNA gene counts in the soil determined by qPCR. Such an approach to determine cell numbers based on 16S operon abundances coupled with taxa specific rRNA operons per cell has been suggested earlier (Fogel *et al.* 1999, Lee *et al.* 2009) but was rarely applied and rather only at higher phylogenetic levels (Carmichael *et al.* 2013, Savichtcheva *et al.* 2011, Trias *et al.* 2012).

Several of the identified rhizodeposit utilisers were highly abundant in summer at the root surface and in the rhizosphere but were almost absent in bulk soil samples. Also, estimated cell numbers of putative rhizodeposit consumers was highest in summer for all rhizosphere samples. *Massilia* spp. appeared to be the most abundant root exudate consumer in summer and their frequency declined markedly in winter. In contrast, bacteria affiliated to *Mucilaginibacter* and *Opitutus* were still abundant and reads from *Ohtaekwangia* even increased at the root surface in winter. Surprisingly, total cell counts of rhizodeposit utilisers increased in winter at root surface. Generally, total 16S rRNA gene abundances in winter exceeded those from root surfaces in summer. Probably, decaying roots provided even more substrates to soil microbes than rhizodeposits. When the maize roots decayed in winter, also rhizodeposit utilisers appeared to be saprophytically involved. Both *Mucilaginibacter* and *Ohtaekwangia* spp. also incorporated litter carbon in the microcosm experiments, and a saprophytic lifestyle is often observed for many rhizobacteria; even for otherwise symbiotic rhizobia (Sadowsky *et al.* 2006).

Cell numbers of identified glucose utilisers were highest for the rhizosphere in summer, but the differences to bulk soil and winter root and rhizosphere samples was less prominent. Glucose consumers were also abundant in bulk soil, although glucose and other soluble sugars are scarce in soil. This further confirms the assumption that identified glucose consumers like *Arthrobacter* spp. may actually be mainly involved in degradation of older SOM via priming effects in the field (Dungait *et al.* 2013). *Arthrobacter* spp. and *Pseudomonas* spp. can degrade recalcitrant organic compounds such as polycyclic aromatic hydrocarbons (PAHs) (Niepceron *et al.* 2010, Thion *et al.* 2012) and bacteria

from both genera are suspected to degrade recalcitrant compound in the presence of easy degradable substances (Thomas *et al.* 2013, Thomas *et al.* 2011).

Unexpectedly, key litter degraders from the SIP experiment were not very abundant in bulk soil, but only at the root surface in winter. Here, as already mentioned, *Mucilaginibacter* spp. and *Ohteakwangia* spp., but also *Flavobacterium* spp. and unclassified *Polyangiaceae* were frequent. Potentially, these bacteria mostly utilised soluble substrates in litter degradation. Except for members of the *Polyangiaceae*, none of these genera are known to degrade cellulose. Moreover, the experimentally identified cellulolytic degraders *Cellvibrio* spp, *Cytophaga* spp., *Kitasatospora* and other unclassified *Streptomycetaceae* were not very abundant in the field.

However, other bacteria affiliated to taxa with cellulolytic abilities were quite frequent at the root surface in the field in winter. These were bacteria affiliated to *e.g. Streptomyces, Rhizobium, Bradyrhizobium, Variovorax, Pseudomonas*, and *Actinoplanes* spp., as well the *Micromonosporaceae* (Ghio *et al.* 2012, Lynd *et al.* 2002, Sadowsky *et al.* 2006, Trujillo-Cabrera *et al.* 2013). Still, these were not labelled in our experiments, probably due to distinct competitive conditions. Especially the rhizobacteria affiliated to *Streptomyces, Rhizobium, Bradyrhizobium* and *Pseudomonas* were already abundant at root surface but not in the bulk soil which was used for the microcosm experiment. Therefore, the putative importance of the identified cellulolytic bacteria cannot be ultimately defined for the field.

Vice versa, especially abundant bulk soil taxa like *Bacillus* spp., the *Acidobacteria* subgroup 4 and 6, *Nitrospirales* spp. and unclassified *Rhizobia* were not ^{13}C labelled in the SIP experiments. However, because of their high abundance *in situ* they are prone to have some share in the carbon turnover in the soil. In the experiments, only fresh carbon inputs were investigated. In contrast, bacteria within such bulk soil habitats can be expected to mainly thrive on older and more recalcitrant SOM. They also have effective persistence strategies, such as the spores formed *e.g.* by *Bacillus* spp, and which might have accumulated over time.

All in all, this comprehensive investigation of key bacterial food web components revealed a strong involvement of bacteria for all plant-derived substrates in the soil. Identified food web members degrading different substrates were not distributed equally in the soil, but were generally highly abundant in the rhizosphere as well as on living and dead roots. This indicates, that carbon fluxes in soil microbial food webs are not as clearly attributed to taxonomic groupings as suggested by the theory of distinct ‘energy channels’. Rather, respective distinctions are much more pronounced in space and time. Substrates from

different sources (mainly rhizodeposits, plant residues and recalcitrant SOM) are degraded by specific microbial populations within the overall microbial community. And, as I postulated in my second hypothesis, specific members of the soil food web exhibited clear spatial and seasonal variance both in distribution and abundance. Therefore, future soil food web models should implement specific and functionally defined microbial populations with spatio-temporal dynamics instead of overall biomass estimations.

4.4. Mobilisation and transport of soil bacteria by seepage water

In this last part of my thesis, I investigated whether bacteria from natural soil microbial communities are mobilised selectively by seepage water, the prime mechanism of vertical transport into subsoils (Balkwill *et al.* 1998). Furthermore, the contribution of transported bacteria on total carbon flux into subsoil was assessed. The Fresh (24 h) lysimeter water from two depths (plough layer, subsoil), soil from the same depths as well as top soil, rhizosphere and the root surface of a remaining root stalk were sampled. Data relate to only one time point of sampling during late winter, were the biological activity in soil is low. Therefore, this study provides only a first insight into possible mechanisms of selective vertical bacterial mobilisation under natural conditions, with a focus on an agricultural soil and seepage water collected in the fallow season. Nevertheless, probably already these initial observations can be seminal to improve our understanding of the vertical transport of natural microbial populations in soil.

4.4.1. Selective mobilisation and transport of bacterial lineages

Bacterial communities transported by seepage water were distinct, especially from bulk soil communities, indicating that transported bacteria were not merely a subset of the total soil microbiota. Rather, bacteria appeared to be mobilised selectively, thus confirming our initial hypothesis. Taxa within the *Bacteroidetes*, *Beta-* and *Alphaproteobacteria*, especially those abundant on the root surface, were transported preferentially, in contrast to *Actinobacteria*, *Firmicutes* and *Acidobacteria*. Bacteria from those phyla were hardly detected in lysimeter water, although abundant in bulk soil. Despite intensive literature research, no other study was found directly demonstrating such a selective mobilisation of natural microbial communities in soil.

In contrast, mobilisation and transport of pathogens from manure or artificial amendments have been intensively investigated (for a comprehensive review see Bradford et al., 2013). Mechanisms of transport observed here should also be valid for native soil bacteria.

Mobilisation of bacteria added to the top soil in unsaturated media is generally impeded by straining because of pore size filtering and interactions with the soil matrix (Sen 2011). Therefore, bacteria are mostly transported by sporadic events with excessive seepage water like heavy rain or snow melt (Jiang *et al.* 2010, Natsch *et al.* 1996). Then, quasi-saturated conditions prevail and water flux is dominated by preferential flow capable of flushing bacteria through macropores, i.e. wormholes, root channels and cracks (Bech *et al.* 2011, Unc *et al.* 2004). These mechanisms can be assumed to be even more relevant at our field site considering the highly condensed plough layers.

The bacterial community from root surface samples was clearly most similar to the lysimeter microbiota. Therefore, we conclude that a significant amount of transported bacteria may actually have been mobilised by water flow along root channels. In disturbed soils like our agricultural field, root channels can form the majority of macropores (Ghestem *et al.* 2011). In winter, roots shrink and decay and give even more space for water flow. Besides being the main flow route of seepage water, root channels harbour high amounts of bacteria in comparison to the surrounding soil and are known to be 'hot spots' of bacterial activity (Bundt *et al.* 2001, Vinther *et al.* 1999). Such macropores feature relatively high nutrient supply from decaying roots and washed out carbon compounds. One may speculate that microbial communities in the bulk soil could be better protected against mobilisation by seepage water, as they often reside within micropores or are attached to soil particles.

Yet, bacterial taxa from lysimeter samples were not of totally equal abundance as in root surface samples. Therefore also other mechanisms may influence bacterial mobilisation. For example *Actinobacteria*, although abundant on the root surface, were not noticeably transported, which could be ascribed to the branched mycelia many *Actinobacteria* form in their active state (Balkwill *et al.* 1998). Spores of *Actinobacteria*, however, have been found in high abundances in deeper soil layers under high recharge, indicating elevated transport by seepage water (Balkwill *et al.* 1998). Generally, hydrophobicity, surface charge, size and cell structure are features known to influence mobilisation rates of bacteria (Bradford *et al.* 2013, Gargiulo *et al.* 2008). How these cell properties specifically affected mobilisation in our study cannot be predicted, as these characteristics are not only species-specific, but also vary with growth state and physical condition (Foppen *et al.* 2006).

Nevertheless, we observed that some bacteria were more readily mobilised than others. Although quite rare in all soil and root surface biota, some bacteria were quite abundant in lysimeter samples, *e.g.* populations within the unclassified *Bacteroidetes*, *Opitutaceae*,

Legionellaceae and *Moraxellaceae*. For *Legionella* spp., a life cycle with sessile biofilm and mobile swarming cells is known (Declerck 2010), and boosts of Legionella infections have been related to heavy rain falls (Fisman *et al.* 2005, Hicks *et al.* 2007). This and their relatively high abundance in lysimeter samples indicate that for some bacteria, transport by seepage water could be even a key strategy for distribution.

Our conclusions are largely based on relative T-RF and sequence read abundances. As discussed above, the congruence of both data sets allows semi-quantitative interpretation and I show that bacterial community distinctions via fingerprinting and amplicon sequencing yielded very similar results in PCA biplots. Many important bacterial taxa, discriminative for a given compartment, were assigned to defined T-RFs of similar discriminative character, which strengthens my overall interpretation of the pyrotag data.

4.4.2. Bacterial contribution to vertical carbon flux

The author is aware of the many assumptions used in the estimation of bacterial cellular carbon content in seepage water. Yet, data handling was chosen carefully and the consistent results between the two estimation approaches confirm at least the correct magnitude of the resulting interpretation. More direct measures like cell counting or chloroform-fumigation-extraction (Daims *et al.* 2001, Vance *et al.* 1987) were not feasible in our study because of the small amount of water from the lysimeters and low cell densities therein. Despite large amounts of seepage water formed upon the event, only a fraction of the total precipitation (42 % in 35cm; 6 % in 65 cm) reached the lysimeters. This may be caused by (a) evapotranspiration, (b) bypassing of lysimeters by macropore flow, (c) lateral interflow on top of the compacted plough-layer and (d) to a lesser extent, saturation of the lysimeter and the potential bypassing as a consequence of excess water saturation. Nevertheless, 42 % collection of the total precipitation at 35 cm depth is in good agreement with measurements from comparable filed sites.

Organic carbon content in seepage water was measured from lysimeter samples prior and after sampling of bacteria, but within the same weather event of rain and snowmelt. As carbon content was very reproducible in those samples, I confidently assume the same amount of carbon in the samples analysed for bacterial communities. In contrary to my third hypothesis, bacterial biomass appeared to contribute only marginally to carbon transported to deeper soil zones. We initially assumed much higher proportions of bacterial carbon because of the relevance of colloidal carbon reported in other studies (Gao *et al.* 2006, Martins *et al.* 2013, Totsche *et al.* 2007) and the overlapping size fractions between

colloids (here: 0.35 to 1 µm) and soil bacteria (ca. 0.2 to 1 µm). Partly, this discrepancy may be attributed to the fact that samples were collected after several days of snowmelt and subsequent rain. Potentially, the biggest load of bacterial cells mobilisable by the event had already been washed out. It has been reported that amounts of mobilised organic carbon and colloids are highest after long dry weather conditions followed by excessive rain and seepage water (Totsche *et al.* 2007). So, seasonal and also time-resolved contribution of bacterial biomass to vertical carbon transport remains to be elucidated.

Still, bacteria remain at least indirectly important for carbon transport into deeper soil layers. Microbes turn over 85-90 % of soil organic carbon (Ekschmitt *et al.* 2008) and up to 80 % of SOM can be derived from dead microbial biomass (Liang *et al.* 2011b). Therefore, bacteria and fungi determine the composition of soil organic carbon and all of its properties relevant for mobilisation, including size, hydrophobicity and charge. Especially fragments from cell walls, proteins and peptides interact strongly with soil particles (Fan *et al.* 2004, Kögel-Knabner 2002, Miltner *et al.* 2012) implicating enhanced immobilisation of those compounds.

As depth and vertical transport was rarely considered in soil food webs so far, I aimed here to provide first insights on how inherent soil bacteria are transported by seepage water in general and if this could influence carbon input into deeper soil layers. As the actual carbon flux allocated to transported bacteria seemed very little, transported DOC can be expected to have major influence on microbial activity and carbon turnover in subsoils. Therefore, transported bacterial can potentially be neglected in food web models. Nevertheless, it still needs to be clarified whether the bacteria from upper soil layers actively add to the carbon turnover in subsoils, and if they have any impact on the composition on the subsoil community.

4.5. Suggestions for improved implementation of bacteria in soil food web models

My work further contributes to the general perspective that the majority of bacteria are inactive or dormant in bulk soil and especially the sub-soil. In contrast, rhizosphere and root habitats comprise a very active bacterial community. Here, specific rhizodeposit consumers are highly abundant in summer, switching with litter degraders in winter. Our findings allow for two major conclusions: first, specific bacterial taxa are involved in all degradation processes of plant-derived substrates in soils. Second, active bacterial populations in the soil food web are dynamic in time and space. This already illustrates that mere total biomass measurements are not sufficient for the integration of bacterial

populations into current food web models. Especially not if determined from bulk soil suspensions (Holtkamp *et al.* 2008), while many bacteria are attached to soil particles.

Also the concept of distinct bacterial and fungal energy channel seems to be disconnected from reality, not least because the notations of these channels seem to vary between reports. Originally, the idea was that bacteria, fungi and roots are main nutrient sources of different secondary consumers but both bacteria and saprophytic fungi use labile and recalcitrant substrates (Hunt *et al.* 1987). When 'energy channels' were explicitly mentioned the first time, it was suggested that there is little trophic interaction among the channels (Moore *et al.* 1988). However, this seems not to hold true for soil food webs (Crotty *et al.* 2011). Later it was postulated that bacteria mainly degrade labile substrates and have higher turnover rates in contrast to fungi, which resulted in the classification of the 'fast' bacterial and 'slow' fungal channel for soil food webs (Moore *et al.* 2003). This separation of fungi and bacteria and their strict assignment to labile and recalcitrant substrates has to be reconsidered as there is actually no field evidence for that, and also my results together with those of other members of the Research Unit FOR-918 are contradicting. Rather, fungi and bacteria seem to compete for both labile and more recalcitrant substrates. The fungi:bacteria ratio seems to be mainly determined by C:N content of the substrate and also soil properties like pH (Strickland *et al.* 2010). Moreover, the degradation of labile and recalcitrant substrates may even be tightly linked by priming effects (Dungait *et al.* 2013).

Conceptual and quantitative models try to generally reflect and predict reality as accurately as possible with a maximum of simplification. Therefore, identification of defined microbial food web members at all possible trophic channels and links as it was done in this thesis and Research Unit, may not be feasible for general food web modelling. For overall food webs more superordinate classifications are needed. Furthermore, even with SIP, the true trophic interactions amongst bacteria (*e.g.* synergism, syntrophy, predation and saprophytic degradation) are often not unambiguously revealed as labelling of specific taxa can be delayed due to slow growth rates or cross-feeding. Here I would like to suggest a more minimalistic approach adapted to the 'fast' and the 'slow' energy channel, but with different distinct categorisation:

Fresh carbon has been reported to be the carbon pool converted most rapidly in soils and even lignin is degraded fast in comparison to aged SOM (Dungait *et al.* 2012). In the rhizosphere and also the detritusphere, bacterial (and probably also fungal) populations are highly active and dynamic (Buée *et al.* 2009). Whereas in bulk soil, dormancy is prevalent

(Lennon *et al.* 2011). Therefore, bacteria could be divided into utilisers of fresh plant-derived substrates in both the rhizo- and the detritussphere; and into utilisers of mostly old SOM, i.e. bacteria from bulk soil. To model their share in carbon pools and flows, rhizosphere and detritusphere bacterial populations should be incorporated into models with seasonal effects. For bulk soil populations, soil horizons need to be considered. For further improvement of accuracy, determination of turnover rates (Rousk *et al.* 2011) or activity could be considered (Christensen *et al.* 1999).

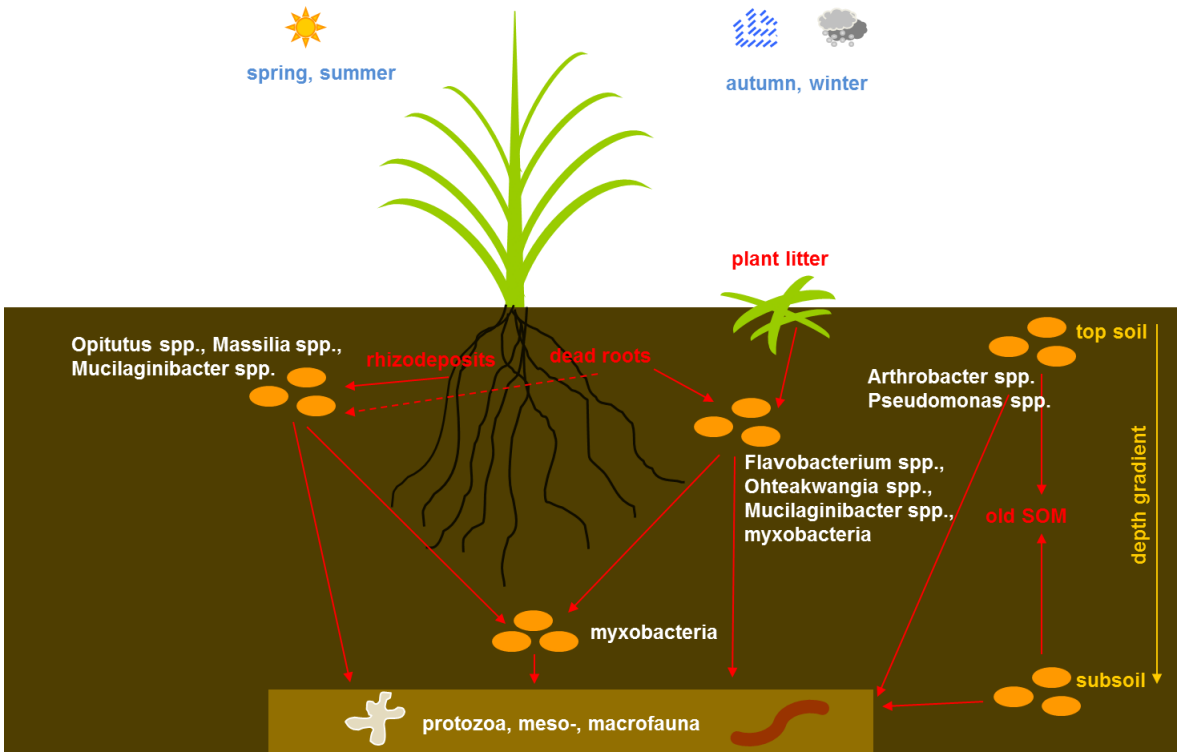

Fig. 25: Proposed concept for an improved implementation of functional bacterial populations in soil food webs with examples from the studies of this thesis.

5. Conclusions

In this thesis, a unique 'field-to-lab-to-field' approach was applied to investigate the bacterial populations distinctively active in a soil food web, as well as their distribution in an agricultural field. Specific bacterial populations were involved in the degradation of the tested substrates (of varying recalcitrance and complexity) and their respective abundance in the field changed with soil compartments and season. Rhizodeposit utilising bacteria (e.g. *Mucilaginibacter* spp., *Massilia* spp., *Opitutus* spp.) identified with SIP were indeed most abundant in rhizosphere samples and at the root surface in summer in the field. Litter degraders, however, were most frequent on dead roots in winter (e.g. *Flavobacterium* spp., *Ohteakwangia* spp., *Mucilaginibacter* spp., myxobacteria). In contrast, identified glucose consumers were also abundant in field bulk soil (e.g. *Arthrobacter* spp., *Pseudomonas* spp.). Generally, cell numbers degreased with depth for all identified bacterial food web members. Those findings are relevant for my first and second hypotheses: Bacteria are not sufficiently represented in current food web models and their role in soil carbon fluxes is in fact not limited to the degradation of labile substrates. Often they are only depicted as one 'black box' just comprising all soil bacterial biomass. Here, I could show that a new concept is needed: Specific bacterial populations, dynamic in space and time, have to be considered to drive carbon fluxes from distinct plant-derived carbon sources in soils. This includes fresh carbon from rhizodeposits and plant litter as well as old SOM. Actually, to my knowledge, this is first time that this has been systematically addressed for such wide range of substrates for one soil and even for several kingdoms as realised in the Research Unit FOR 918, where this work was embedded in.

With my third hypothesis, I expected that subsets of inherent soil bacteria are mobilised and transported selectively by seepage water and that they do contribute markedly to the total carbon washed out into the subsoil. Indeed, the composition of the transported bacterial population resembled mostly that from root surface, indicating major transport selection by preferential flow of seepage water. Unexpectedly, estimated bacterial carbon appeared low, and probably not decisive for subsoil carbon replenishment. Still the impact of those transported bacteria on carbon turnover and subsoil community composition remains to be clarified. With this comprehensive study on specific bacterial members of soil food webs, I could emphasise the persisting demands to regard microbial functional diversity in future soil food webs.

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Appendix

Table A.1: Mean relative T-RF abundances and the respective standard deviation (SD) of the horizontal and vertical soil transects as depicted in figure Fig. 9 (table continues on the next page).

	mean abundance									SD								
T-RF	length [m]						depth [m]			length [m]						depth [m]		
[bp]	0	0.2	1	5	25	125	0.05	0.35	0.65	0	0.2	1	5	25	125	0.05	0.35	0.65
60	2.0	2.0	1.9	1.6	2.1	2.5	1.6	2.1	2.0	0.3	0.3	0.1	1.4	0.6	0.8	0.1	0.6	0.3
61	3.7	3.2	3.8	4.8	4.7	6.2	4.8	2.7	1.7	0.3	0.5	0.5	2.1	1.2	0.1	0.4	0.2	0.2
63	0.0	0.0	0.0	1.7	0.0	0.0	1.7	0.0	1.5	0.0	0.0	0.0	2.9	0.0	0.0	0.1	0.0	0.9
66	0.7	0.7	1.0	0.9	0.5	0.4	0.9	0.6	0.0	0.1	0.0	0.1	0.3	0.4	0.5	0.3	0.5	0.0
69	1.4	1.2	1.5	1.5	0.7	0.6	1.5	1.3	0.7	0.0	0.1	0.3	0.2	0.6	0.7	0.1	0.4	0.2
71	0.5	0.7	0.6	0.5	0.3	0.0	0.5	0.3	0.0	0.0	0.1	0.1	0.4	0.3	0.3	0.1	0.3	0.0
72	0.8	1.1	1.0	1.1	0.6	0.5	1.1	1.6	0.4	0.3	0.2	0.3	0.2	0.5	0.6	0.2	0.7	0.3
74	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.3	0.4	0.0	0.0	0.8
77	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.5	0.0	0.0	0.2	0.0	0.0	0.0	0.1	0.2	0.4
79	0.4	0.0	0.2	0.0	0.1	0.0	0.0	0.2	0.0	0.4	0.0	0.2	0.0	0.1	0.2	0.1	0.2	0.0
81	1.6	2.2	1.6	1.2	1.7	0.8	1.2	0.6	3.8	0.5	1.0	0.8	0.4	0.4	0.6	0.1	0.5	5.0
83	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.0
87	2.0	1.4	1.9	2.1	1.1	1.0	2.1	1.2	0.2	0.6	0.4	0.6	1.0	0.9	1.2	0.4	1.1	0.3
90	1.9	2.7	2.4	1.3	1.4	1.6	1.3	2.6	1.1	0.5	0.4	0.8	1.1	1.2	1.6	1.1	0.5	1.1
91	1.1	0.6	1.3	1.7	1.2	0.9	1.7	2.0	2.4	1.0	1.0	1.2	1.6	1.0	1.2	0.2	0.1	0.1
113	0.3	0.5	0.2	0.0	0.1	0.0	0.0	0.4	0.9	0.2	0.1	0.2	0.0	0.2	0.2	0.3	0.4	0.2
116	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
117	2.3	2.6	2.2	1.5	2.1	1.7	1.5	1.0	0.3	0.4	0.2	0.1	1.3	1.2	1.3	0.2	0.9	0.3
119	0.9	0.7	0.8	0.6	0.3	0.0	0.6	1.2	1.5	0.1	0.1	0.2	0.5	0.4	0.2	0.1	0.5	0.6
122	0.1	0.5	0.3	0.4	0.4	0.3	0.4	0.7	1.0	0.2	0.0	0.3	0.4	0.3	0.4	0.3	0.3	0.1
124	0.7	0.8	0.8	0.6	0.5	0.3	0.6	0.9	0.1	0.2	0.1	0.1	0.5	0.5	0.5	0.2	0.4	0.2
127	1.2	1.2	1.3	1.4	1.5	1.1	1.4	1.0	1.3	0.1	0.1	0.1	0.5	0.5	0.0	0.4	0.2	0.8
128	2.4	2.5	2.3	2.9	2.9	3.6	2.9	2.5	2.8	0.2	0.3	0.1	0.4	0.8	0.8	0.5	0.4	1.1
133	3.1	1.8	2.2	1.6	2.0	2.4	1.6	1.0	0.0	1.5	0.4	0.3	0.3	0.9	0.9	0.9	0.9	0.0
135	0.5	0.8	0.3	0.0	0.1	0.0	0.0	2.2	3.3	0.2	0.2	0.3	0.0	0.1	0.2	0.4	2.2	2.2
137	1.4	1.4	1.1	0.7	0.6	0.3	0.7	1.1	4.2	0.1	0.0	0.1	0.6	0.6	0.4	0.1	0.3	0.9
139	4.5	5.1	4.9	5.4	5.1	5.8	5.4	4.5	6.1	0.4	0.4	0.2	0.3	1.1	1.5	0.5	0.6	0.6
141	1.7	2.9	2.3	2.2	2.3	3.4	2.2	1.9	0.3	0.9	0.5	1.3	0.4	1.7	2.3	0.7	0.9	0.6
143	0.9	0.2	0.6	0.0	0.3	0.4	0.0	0.1	0.1	0.5	0.3	0.3	0.0	0.4	0.5	0.2	0.2	0.2
145	0.7	1.0	0.9	0.8	1.0	1.2	0.8	0.7	0.0	0.3	0.3	0.2	0.7	1.2	1.6	0.3	0.2	0.0
146	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.4
147	5.3	4.7	5.5	6.5	5.6	5.7	6.5	5.5	8.7	0.1	0.4	0.6	2.2	0.4	0.3	0.7	1.3	0.5
149	6.4	5.8	5.9	7.4	6.8	6.1	7.4	5.1	3.8	0.5	0.7	0.9	0.1	2.0	1.5	0.8	1.3	0.3
150	0.3	0.7	0.3	1.4	0.8	0.8	1.4	2.8	0.0	0.3	0.7	0.3	0.6	1.0	1.3	0.5	4.2	0.0
153	0.1	0.0	0.2	0.2	0.2	0.3	0.2	0.0	0.0	0.2	0.0	0.2	0.4	0.4	0.5	0.2	0.0	0.0
154	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0
158	9.3	9.3	9.3	11.4	12.8	14.6	11.4	9.0	6.9	0.8	1.4	0.3	1.3	4.8	5.9	0.9	1.5	1.0
161	1.1	0.7	1.3	1.3	0.7	0.5	1.3	0.7	1.4	0.3	0.0	0.5	0.1	0.6	0.8	0.1	0.1	0.3
163	0.0	0.3	0.1	0.4	0.2	0.0	0.4	0.5	0.4	0.0	0.2	0.2	0.7	0.3	0.3	0.4	0.2	0.7
165	1.1	1.1	1.3	1.5	1.5	0.9	1.5	1.1	1.2	0.1	0.3	0.0	0.2	0.4	0.3	0.3	0.1	0.6
167	2.0	1.9	2.5	2.2	2.1	2.6	2.2	2.4	1.0	0.2	0.2	0.1	0.1	0.8	0.8	0.2	1.0	0.3
169	1.4	1.8	1.3	1.7	1.8	2.5	1.7	1.8	3.6	0.4	0.2	0.3	0.4	0.9	1.2	0.4	0.8	0.2
171	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.6	1.4	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.4	1.0
172	0.6	0.6	0.4	1.0	0.6	0.5	1.0	0.3	0.0	0.1	0.1	0.4	0.2	0.5	0.6	0.4	0.3	0.0
187	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.0	0.2	0.2	0.2	0.0	0.0
193	0.3	0.3	0.3	0.0	0.1	0.0	0.0	0.1	0.0	0.2	0.3	0.3	0.0	0.3	0.3	0.3	0.2	0.0
196	2.7	2.9	2.8	3.4	3.3	3.8	3.4	1.9	0.9	0.5	0.2	0.3	0.4	0.8	0.9	0.9	0.1	0.2
199	1.7	1.7	1.6	2.1	2.0	2.2	2.1	1.0	0.0	0.2	0.1	0.1	0.3	0.4	0.4	0.4	0.4	0.0

APPENDIX

	mean abundance									SD								
T-RF	length [m]						depth [m]			length [m]						depth [m]		
[bp]	0	0.2	1	5	25	125	0.05	0.35	0.65	0	0.2	1	5	25	125	0.05	0.35	0.65
201	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.6	0.3
205	1.3	0.8	1.3	1.3	0.6	0.5	1.3	1.0	0.2	0.4	0.1	0.4	0.4	0.5	0.6	0.1	0.3	0.3
207	0.6	0.0	0.3	0.0	0.1	0.0	0.0	0.1	0.0	0.7	0.0	0.3	0.0	0.2	0.3	0.1	0.2	0.0
223	0.6	0.6	0.6	0.6	0.4	0.0	0.6	0.4	0.7	0.1	0.1	0.1	0.5	0.4	0.4	0.3	0.1	0.3
263	0.7	0.8	1.0	0.5	0.5	0.0	0.5	0.9	0.0	0.1	0.0	0.2	0.5	0.6	0.3	0.8	1.1	0.0
277	0.8	0.6	0.5	0.6	0.4	0.3	0.6	0.3	0.3	0.1	0.1	0.4	0.5	0.3	0.4	0.1	0.3	0.5
280	0.6	0.7	0.6	0.2	0.4	0.0	0.2	0.5	0.0	0.0	0.1	0.0	0.4	0.4	0.2	0.0	0.4	0.0
288	2.3	2.4	1.9	2.5	2.4	3.1	2.5	1.6	2.0	0.7	0.2	0.3	0.3	0.8	1.1	0.7	0.5	0.1
290	1.0	1.0	0.9	1.0	0.6	0.6	1.0	0.7	0.0	0.0	0.1	0.1	0.1	0.5	0.7	0.1	0.2	0.0
294	1.3	1.9	1.5	1.8	1.8	2.5	1.8	2.6	0.9	0.4	0.2	0.4	0.1	0.9	1.2	0.2	2.4	0.0
311	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.1	0.6	0.2	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.0
397	1.2	1.5	1.2	0.5	0.3	0.0	0.5	0.9	1.4	0.2	0.3	0.2	0.4	0.6	0.0	0.2	0.2	1.1
398	0.5	0.4	0.6	0.0	0.4	0.3	0.0	0.4	0.0	0.0	0.4	0.3	0.0	0.4	0.4	0.2	0.3	0.0
401	0.2	0.1	0.1	0.0	0.3	0.0	0.0	0.1	0.2	0.2	0.2	0.2	0.0	0.3	0.3	0.2	0.2	0.4
403	1.0	1.0	1.1	1.4	1.4	1.8	1.4	5.1	0.2	0.1	0.1	0.1	0.5	0.3	0.3	0.5	6.9	0.4
423	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.2	0.3	0.2	0.2	0.0
426	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.2	0.1	0.0	0.0	0.0	0.1	0.0	0.4
428	0.6	0.7	0.7	0.2	0.5	0.4	0.2	0.5	0.0	0.1	0.1	0.1	0.4	0.5	0.5	0.1	0.5	0.0
433	0.8	0.8	0.8	1.1	0.7	0.6	1.1	0.5	0.8	0.0	0.0	0.2	0.5	0.6	0.8	0.1	0.5	0.3
437	0.0	0.0	0.5	0.0	0.6	0.7	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.6	0.8	0.0	0.0	0.0
438	1.3	1.6	1.5	1.4	1.5	0.6	1.4	2.3	3.9	0.1	0.2	0.4	0.2	0.4	0.5	0.2	0.6	1.5
440	0.3	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.3	0.3	0.0	0.0	0.3	0.0	0.2	0.0	0.4
447	1.1	1.2	1.0	1.2	1.5	1.9	1.2	0.6	0.0	0.2	0.1	0.3	0.3	0.3	0.4	0.1	0.5	0.0
455	1.0	1.2	1.1	0.6	0.6	0.4	0.6	0.9	0.0	0.2	0.2	0.2	0.6	0.6	0.4	0.3	0.9	0.0
461	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.3	0.0	0.0
466	0.7	0.8	0.4	0.6	0.5	0.4	0.6	0.4	1.3	0.1	0.1	0.4	0.5	0.5	0.5	0.4	0.4	0.5
468	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3
469	0.4	0.3	0.1	0.0	0.3	0.0	0.0	0.1	0.0	0.1	0.2	0.2	0.0	0.3	0.3	0.2	0.2	0.0
475	0.5	0.6	0.4	0.5	0.4	0.0	0.5	0.1	0.0	0.1	0.1	0.3	0.4	0.4	0.3	0.3	0.2	0.0
477	0.3	0.5	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.2	0.0	0.0	0.0	0.2	0.0	0.0
488	0.4	0.1	0.1	0.0	0.1	0.0	0.0	0.6	4.9	0.1	0.2	0.2	0.0	0.1	0.2	0.1	0.4	5.4
490	2.2	2.4	2.1	2.0	3.2	4.2	2.0	2.0	2.3	0.2	0.7	0.4	0.2	1.9	2.4	0.7	1.0	0.8
492	0.5	0.1	0.6	0.0	0.1	0.0	0.0	1.6	3.6	0.0	0.2	0.2	0.0	0.1	0.2	0.4	0.5	1.2
494	0.3	0.1	0.0	0.0	0.1	0.0	0.0	0.4	0.0	0.3	0.2	0.0	0.0	0.2	0.2	0.2	0.3	0.0
497	0.6	0.6	0.7	0.3	0.5	0.3	0.3	0.4	0.3	0.1	0.1	0.1	0.5	0.4	0.4	0.4	0.5	0.6
504	1.0	1.3	1.0	1.1	1.2	0.4	1.1	0.7	0.0	0.3	0.1	0.1	0.1	0.5	0.7	0.1	0.2	0.0
508	1.1	1.3	1.1	1.1	1.3	1.9	1.1	1.3	1.3	0.3	0.1	0.1	0.1	0.4	0.5	0.3	0.7	0.5
517	0.6	0.5	0.5	0.6	0.6	0.4	0.6	0.0	0.2	0.0	0.1	0.1	0.5	0.5	0.5	0.0	0.0	0.3
519	0.1	0.0	0.5	0.0	0.3	0.4	0.0	0.4	0.0	0.2	0.0	0.2	0.0	0.5	0.6	0.0	0.3	0.0
526	1.7	2.0	1.8	1.2	1.7	2.4	1.2	1.4	1.3	0.4	0.3	0.1	1.0	1.0	1.4	0.5	0.5	0.3
539	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.4	0.0	0.0	0.0	0.0	0.2	0.3	0.2	0.4	0.7
541	0.3	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.3	0.0	0.4	0.0	0.3	0.3	0.1	0.0	0.0
583	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
636	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.3	0.3	0.0
668	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
670	1.3	1.6	1.2	0.9	1.3	0.3	0.9	0.9	0.0	0.4	0.2	0.2	0.8	0.6	0.7	0.2	0.1	0.0

Table A.2: Total 16S rRNA gene abundances in field bulk soil samples quantified with qPCR as depicted in Fig. 14A.

		top soil	plough layer	subsoil
mean	maize May '09	8.54E+09	6.68E+09	5.11E+08
	maize July '09	1.12E+10	1.01E+10	4.82E+09
	maize Sep. '09	4.35E+09	1.06E+09	4.31E+08
	corn maize Dec. '09	5.82E+09	2.37E+09	8.10E+08
	fodder maize Dec. '09	4.89E+09	2.33E+09	4.61E+08
	corn maize July '10	1.29E+10	6.86E+09	1.16E+09
	fodder maize July '10	3.31E+09	1.01E+09	3.70E+08
SD	maize May '09	3.41E+09	4.45E+09	1.45E+08
	maize July '09	5.32E+09	4.06E+09	3.42E+09
	maize Sep. '09	1.97E+09	3.83E+08	1.86E+08
	corn maize Dec. '09	1.70E+09	5.08E+08	1.51E+08
	fodder maize Dec. '09	1.63E+09	6.72E+08	2.48E+08
	corn maize July '10	4.73E+09	3.33E+09	5.90E+08
	fodder maize July '10	1.34E+09	3.35E+08	1.17E+08

Table A.3: Total 16S rRNA gene abundances quantified with qPCR as depicted in Fig. 14B and C.

		rhizosphere	root surface
mean	July 2009	2.94E+10	7.84E+09
	Sep. 2009	3.66E+09	2.29E+09
	Dec. 2009	1.32E+10	3.41E+10
	July 2010	1.36E+10	1.14E+10
SD	July 2009	5.50E+09	4.54E+09
	Sep. 2009	1.65E+09	6.28E+08
	Dec. 2009	5.31E+09	1.42E+10
	July 2010	7.11E+09	6.30E+09

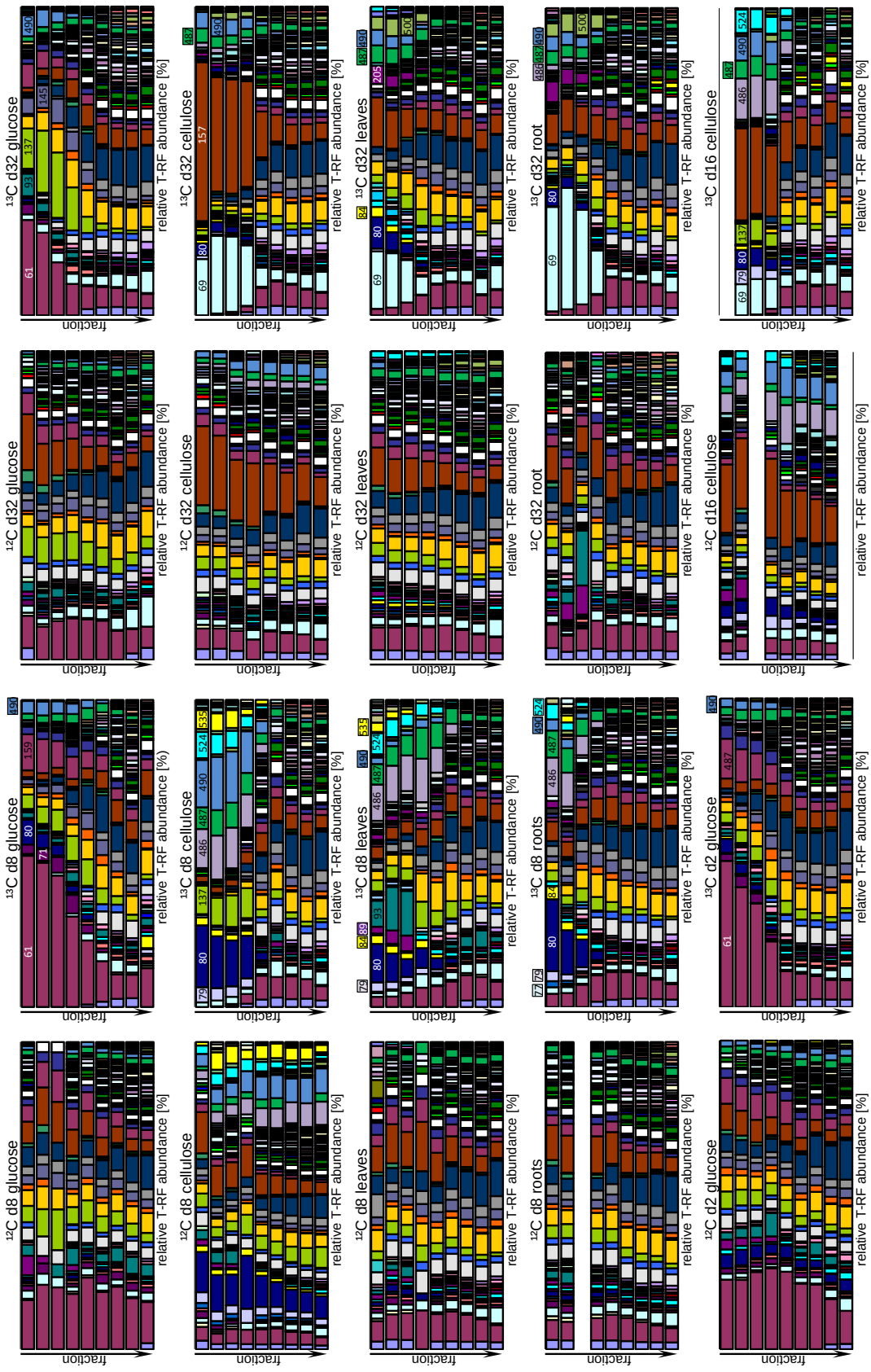


Fig. A.1: Bar plots of the 16S rRNA T-RFLP fingerprints from the detritus SIP experiments. Arrows indicate fractions from 'heavy' (base) to 'light' (point) of the ^{14}C and ^{13}C gradients. d2, d8, d16 and d32 are the sampling timepoints 2, 8, 16 and 32 days after the experiment started.

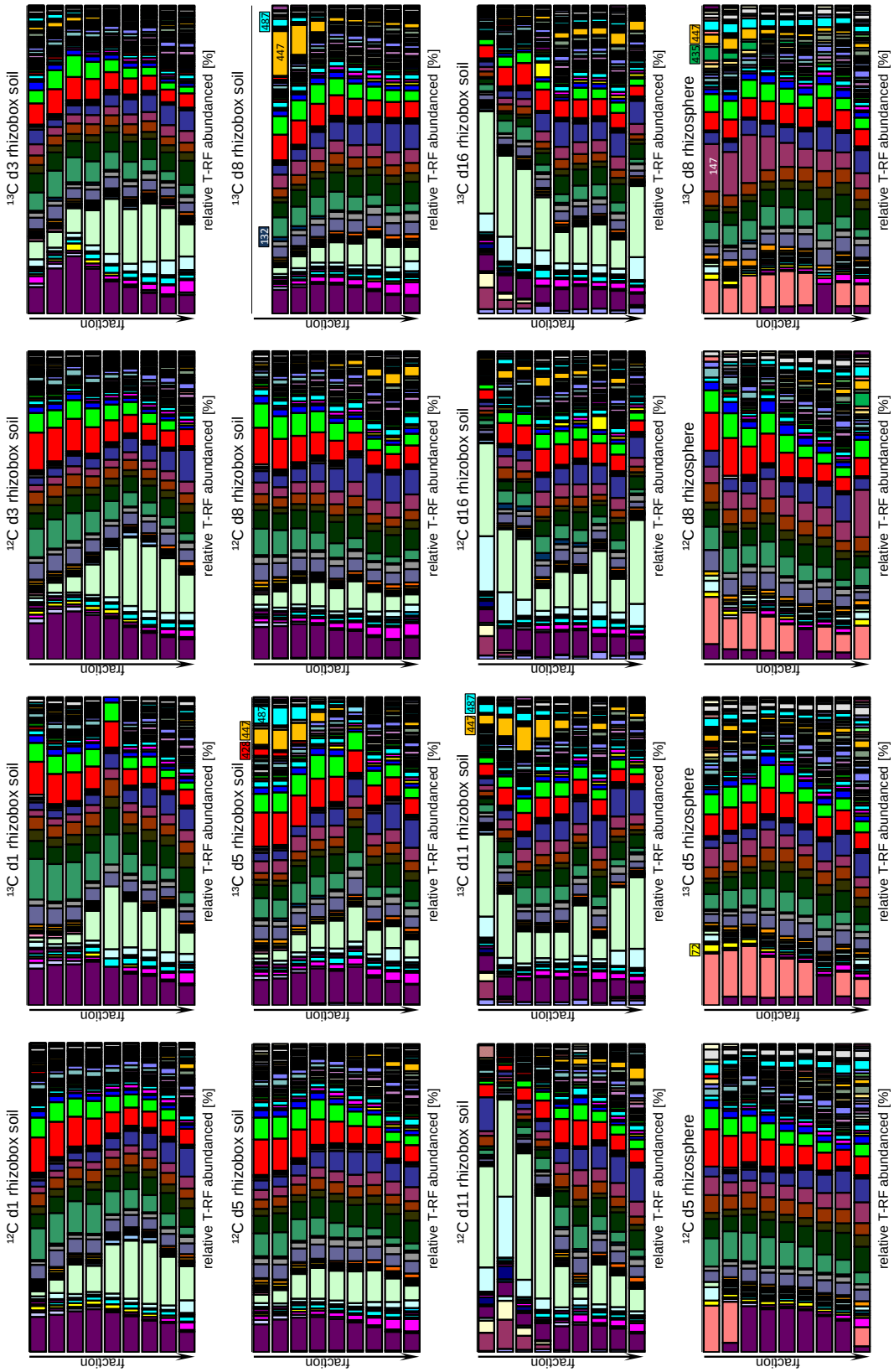
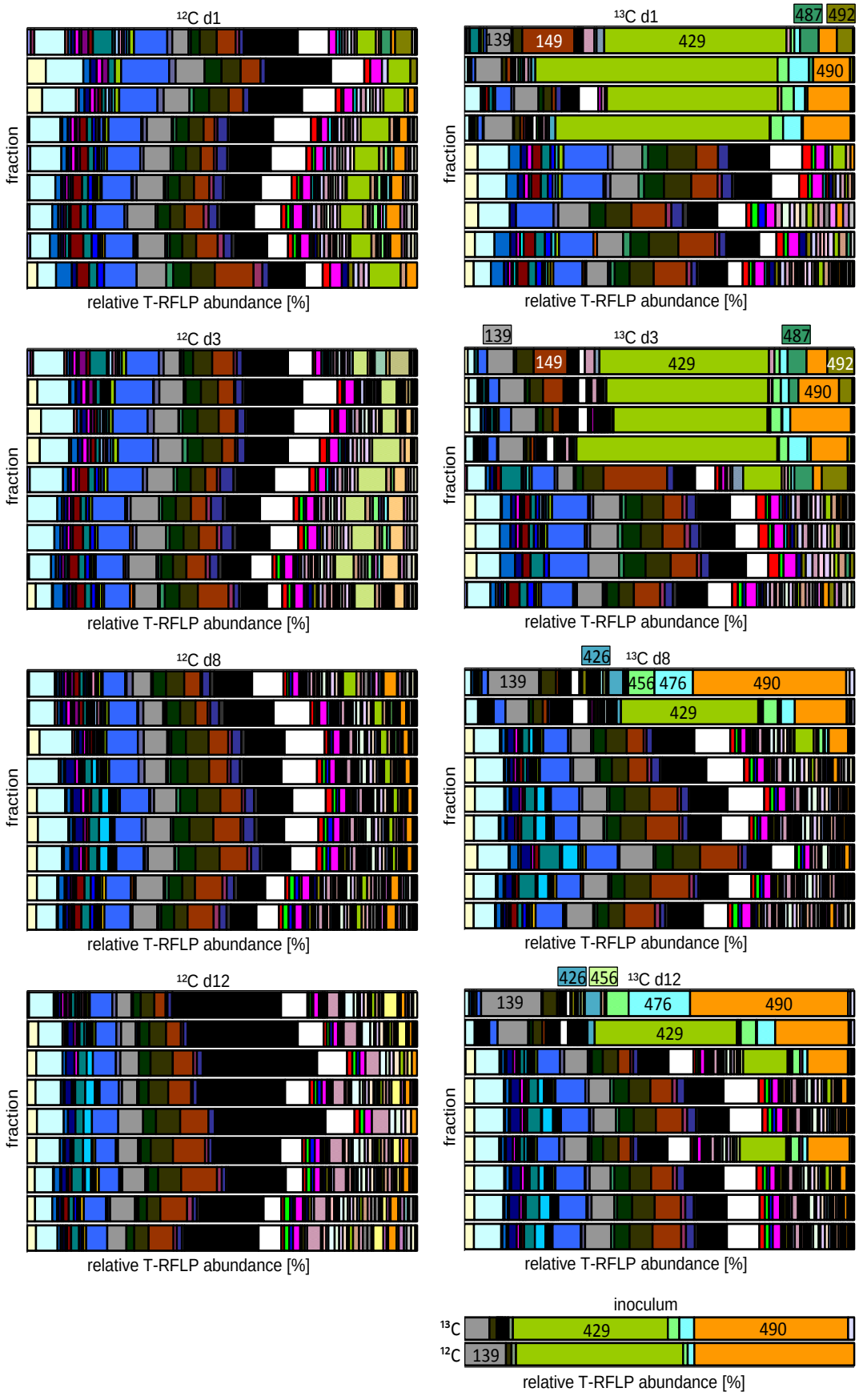


Fig. A.2: Bar plots of the 16S rRNA T-RFLP fingerprints from the rhizosphere SIP experiment. Arrows indicate fractions from 'heavy' (base) to 'light' (point) of the ^{12}C and ^{13}C gradients. d1, d3, d5, d8, d11 and d16 are the sampling time points 1, 3, 5, 8, 11 and 16 days after the experiment started.

Fig. A.3: Bar plots of the 16S rRNA T-RFLP fingerprints from the biomass SIP experiment. Arrows indicate fractions from 'heavy' (base) to 'light' (point) of the ^{12}C and ^{13}C gradients. d1, d3, d8, and d12 are the sampling time points 1, 3, 8, and 12 days after the experiment



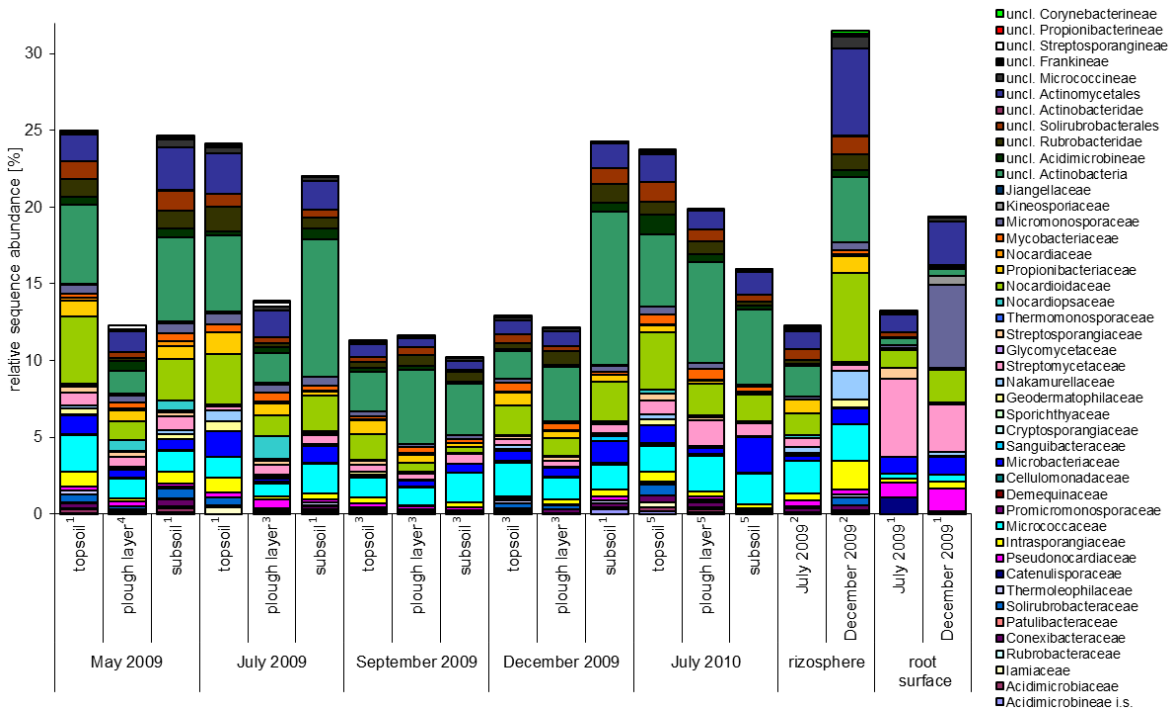


Fig. A.4: Relative pyrotag sequence abundances of families from the phyla *Actinobacteria* from the field samples. Superscripts denote different runs of pyrosequencing.

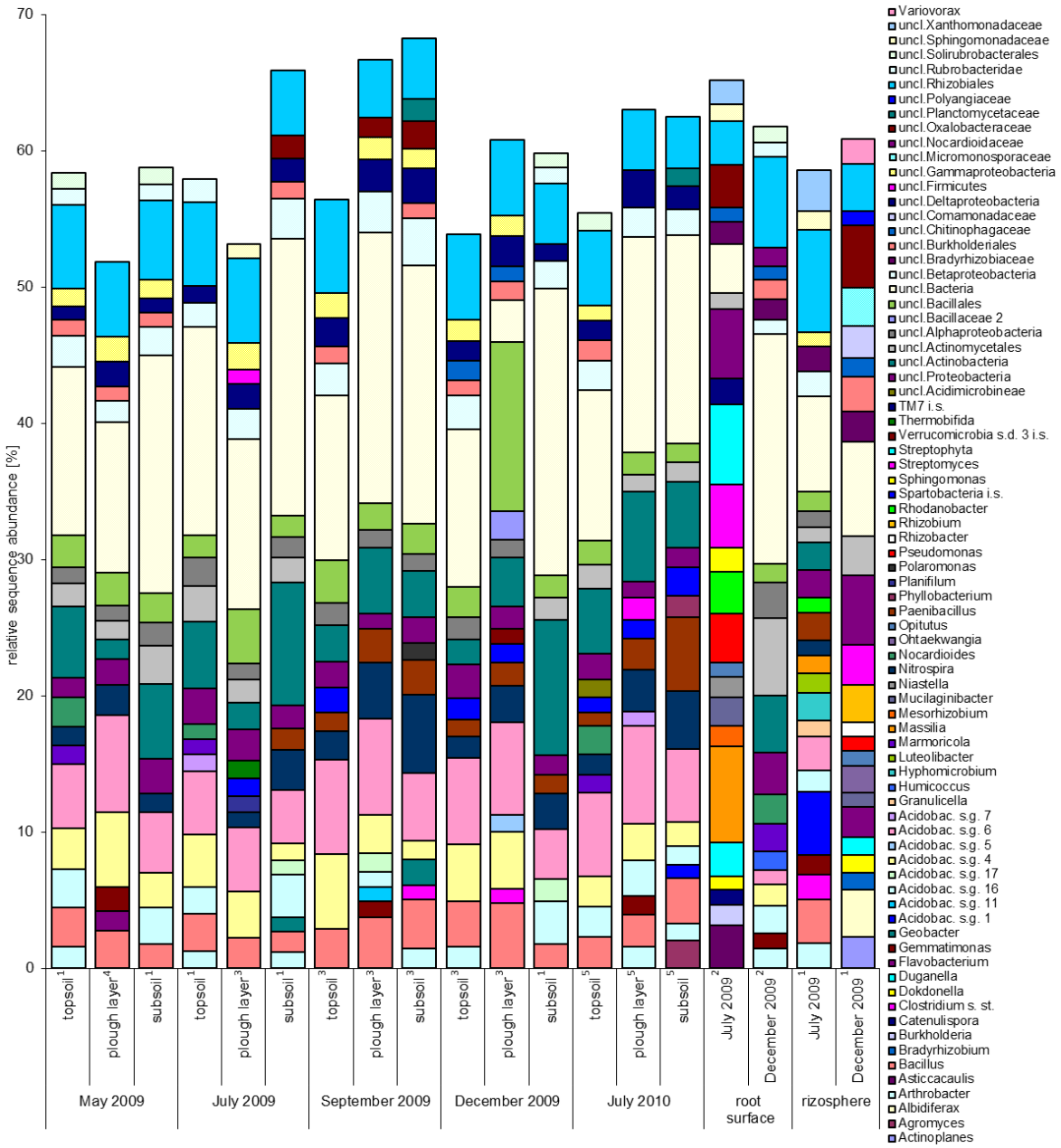


Fig. A.5: Relative pyrotag sequence abundances of most abundant (> 1 %) bacterial taxa at genus level from the field samples. Superscripts denote different runs of pyrosequencing.

Table A.4: Estimated average 16S rRNA gene copy number per cell (c/c) for the most important taxa (representing over 90 % of all obtained pyrotag sequences) in the lysimeter and soil samples from result part 4. Average rrn operon copy number per cell was inferred from the IMG database (Markowitz et al. 2012) from all genomes available within a given taxonomic unit (i.e., family or likewise; table continues on the next page).

	c/c	L38	L65	RS	Rh	B10	B50	B70
Acetobacteraceae	3.07	n.i.	n.i.	n.i.	n.i.	0.32	n.i.	n.i.
*Actinosynnemataceae**	2.58	n.i.	n.i.	n.i.	n.i.	0.36	n.i.	n.i.
Alcaligenaceae	2.47	n.i.	n.i.	0.51	n.i.	n.i.	n.i.	n.i.
Anaerolineaceae	2	n.i.	n.i.	n.i.	n.i.	0.38	1.09	0.66
Bacillaceae	6.55	n.i.	n.i.	n.i.	8.59	3.6	4.7	6.67
Bradyrhizobiaceae	1.32	2.01	12.5	3.05	2.1	1.5	0.93	0.8
Burkholderiaceae	3.1	n.i.	0.83	n.i.	n.i.	n.i.	n.i.	n.i.
Burkholderiales i.s.	1.67	2.31	3.3	3.13	1.2	0.72	0.37	0.59
Caldilineaceae	2	n.i.	n.i.	n.i.	n.i.	0.3	n.i.	n.i.
Caulobacteraceae	1.75	1.08	0.87	1.92	0.75	0.46	n.i.	n.i.
Chitinophagaceae	6	1.98	2.33	5.34	5.52	3.28	1.41	1.4
Clostridiaceae	7.13	n.i.	n.i.	n.i.	1.12	0.42	0.29	n.i.
Comamonadaceae	2.42	3.97	17.4	7.09	1.92	1.22	1.94	5.65
Coxiellaceae	1.17	0.99	n.i.	n.i.	n.i.	0.66	0.29	n.i.
Cytophagaceae	2.72	n.i.	n.i.	0.64	1	0.42	n.i.	n.i.
Enterobacteriaceae	5.51	n.i.	n.i.	0.94	0.3	n.i.	n.i.	n.i.
Flavobacteriaceae	2.2	3.78	n.i.	11.2	1.35	0.78	0.93	0.57
Gammaproteobacteria i.s.	1.54	0.74	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Gemmatimonadaceae	1	0.51	n.i.	n.i.	0.97	1.5	1.57	1.16
Geobacteraceae	1.15	n.i.	n.i.	n.i.	n.i.	n.i.	0.37	0.76
Acidobacteria s.g. 1*	1.43	n.i.	n.i.	n.i.	1.6	1.22	0.82	0.95
Acidobacteria s.g. 11*	1.43	n.i.	n.i.	n.i.	n.i.	0.4	1.12	0.87
Acidobacteria s.g. 16*	1.43	n.i.	n.i.	n.i.	0.57	0.38	0.93	0.73
Acidobacteria s.g. 17*	1.43	n.i.	n.i.	n.i.	n.i.	n.i.	0.4	0.28
Acidobacteria s.g. 18*	1.43	n.i.	n.i.	n.i.	n.i.	n.i.	0.35	n.i.
Acidobacteria s.g. 22*	1.43	n.i.	n.i.	n.i.	n.i.	n.i.	0.32	n.i.
Acidobacteria s.g. 25*	1.43	n.i.	n.i.	n.i.	n.i.	n.i.	0.42	0.45
Acidobacteria s.g. 3*	1.43	n.i.	n.i.	n.i.	0.72	0.86	0.45	n.i.
Acidobacteria s.g. 4*	1.43	n.i.	n.i.	n.i.	4.07	5.95	5.71	2.25
Acidobacteria s.g. 5*	1.43	n.i.	n.i.	n.i.	0.37	0.64	1.27	0.59
Acidobacteria s.g. 6*	1.43	0.48	n.i.	n.i.	1.55	5.29	5.87	4.28
Acidobacteria s.g. 7*	1.43	n.i.	n.i.	n.i.	n.i.	0.44	1.12	0.8
Hyphomicrobiaceae	1.3	n.i.	n.i.	4.52	1.45	1.08	0.88	0.97
Intrasporangiaceae	1.86	n.i.	n.i.	n.i.	0.7	0.32	n.i.	n.i.
Legionellaceae	2.8	2.74	1.91	n.i.	n.i.	0.3	n.i.	n.i.
Methylophilaceae	2.21	6.02	10.7	3.07	0.72	0.5	n.i.	n.i.
Microbacteriaceae	1.67	n.i.	0.76	n.i.	0.9	0.76	n.i.	0.47
Micrococcaceae	2.68	n.i.	n.i.	1.19	2.67	0.88	1.43	2.88
Micromonosporaceae	3.73	n.i.	n.i.	1.4	n.i.	0.64	n.i.	n.i.
Moraxellaceae	3.18	4.27	n.i.	1.15	n.i.	0.36	1.96	n.i.
Mycobacteriaceae	1.25	n.i.	n.i.	n.i.	0.35	n.i.	n.i.	n.i.
Nakamurellaceae	1.50	n.i.	n.i.	n.i.	0.55	0.3	n.i.	n.i.
Nitrosomonadaceae	1	n.i.	n.i.	n.i.	0.3	0.68	n.i.	n.i.
Nitrospiraceae	1.25	n.i.	n.i.	n.i.	1.02	1.84	5.89	5.49
Nocardioidaceae	3	n.i.	n.i.	0.77	2.25	1.34	0.66	1.3
OD1 genera i.s.*	3.21	0.81	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Opitutaceae	1	3.5	n.i.	0.6	0.57	0.68	n.i.	n.i.
Oxalobacteraceae	3.17	10.2	11.9	8.09	3.22	0.52	1.75	3.5
Paenibacillaceae	7.66	n.i.	n.i.	n.i.	1.97	1.34	1.59	3
Phyllobacteriaceae	1.72	n.i.	n.i.	0.81	1.05	0.44	0.35	0.95

Planctomycetaceae	2.78	n.i.	n.i.	n.i.	2.17	2.52	2.92	2.34
Planococcaceae	3	n.i.	n.i.	n.i.	0.82	0.42	0.45	n.i.
Polyangiaceae	4.00	n.i.	n.i.	n.i.	n.i.	0.3	n.i.	n.i.
Propionibacteriaceae	2.29	n.i.	n.i.	n.i.	0.6	0.4	0.29	n.i.
Pseudomonadaceae	2.71	5.28	1.15	4.58	0.65	1.02	0.93	4.75
Rhizobiaceae	2.19	n.i.	n.i.	3.6	0.75	n.i.	n.i.	n.i.
Rhodocyclaceae	2.69	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	0.83
Rhodospirillaceae	3.63	n.i.	n.i.	n.i.	n.i.	0.44	n.i.	0.31
Sinobacteraceae	2.75	n.i.	n.i.	n.i.	n.i.	0.44	n.i.	n.i.
Sphingobacteriaceae	2.81	6.66	6.22	6.69	1.3	0.6	n.i.	n.i.
Sphingomonadaceae	2.13	4.4	7.02	2.13	1.82	2.08	1.01	1.23
Streptomycetaceae	5	n.i.	n.i.	1.24	0.42	0.3	n.i.	0.66
Verrucomicrobia s.d. 3	1	n.i.	n.i.	n.i.	1.57	2.5	1.65	0.59
Thermoactinomycetaceae	5.5	n.i.	n.i.	n.i.	0.87	0.34	n.i.	n.i.
TM7 i.s.	1	1.15	n.i.	1.32	0.8	0.86	n.i.	n.i.
unclass. *Actinobacteria*	2.58	n.i.	n.i.	n.i.	0.8	0.98	1.65	1.92
unclass. *Actinomycetales*	2.69	n.i.	n.i.	n.i.	0.75	0.7	0.82	0.54
unclass. *Alphaproteobacteria*	2.09	2.14	0.8	0.51	1.02	1.76	1.14	0.83
unclass. *Bacillales*	5.53	n.i.	n.i.	n.i.	1.1	0.44	0.42	0.43
unclass. Bacteria	3.21	10	4.59	3.98	9.36	11	16.2	15.3
unclass. *Bacteroidetes*	2.31	4.54	4.8	n.i.	n.i.	0.86	0.35	n.i.
unclass. *Betaproteobacteria*	2.81	0.76	n.i.	n.i.	1.37	3.06	2.79	2.34
unclass. *Burkholderiales*	2.79	n.i.	n.i.	0.85	0.77	1.32	1.14	0.97
unclass. *Clostridiales*	4.43	n.i.	n.i.	n.i.	0.35	n.i.	n.i.	n.i.
unclass. *Deltaproteobacteria*	2.23	0.71	n.i.	n.i.	1.05	1.32	2.6	1.92
unclass. *Firmicutes*	4.23	n.i.	n.i.	n.i.	0.42	n.i.	n.i.	n.i.
unclass. *Gammaproteobacteria*	4.26	4.2	2.02	1.64	1	2.16	1.14	0.97
unclass. *Myxococcales*	2.36	n.i.	n.i.	n.i.	3.49	0.3	0.32	n.i.
unclass. *Proteobacteria*	3.26	2.7	0.94	1.85	1.25	1.36	1.46	0.83
unclass. *Pseudomonadales*	2.9	0.69	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
unclass. *Rhizobiales*	2.08	0.62	n.i.	2.21	3.49	4.37	4.04	3.88
unclass. *Solirubrobacterales*	1.00	n.i.	n.i.	n.i.	0.47	n.i.	n.i.	n.i.
unclass. *Sphingobacteriales*	2.81	n.i.	n.i.	1.72	0.35	1.08	0.37	0.38
unclass. *Verrucomicrobia*	1.28	n.i.	n.i.	n.i.	0.27	0.34	n.i.	n.i.
Verrucomicrobiaceae	2.25	0.62	n.i.	n.i.	0.75	0.36	n.i.	n.i.
WS3 genera i.s.*	3.21	n.i.	n.i.	n.i.	n.i.	n.i.	0.66	0.45
Xanthomonadaceae	2.07	0.95	n.i.	2.39	1.8	2.46	0.5	0.78

* – no affiliated genomes available. Operon copy number was estimated from next higher phylogenetic level.

n.i. – not included in 90% threshold.

i.s. – incertae sedis.

Publications and authorship clarifications

Accepted and submitted

1. Scharroba, A., **Dibbern, D.**, Hünninghaus, M., Kramer, S., Moll, J., Butenschoen, O., Bonkowski, M., Buscot, F., Kandeler, E., Koller, R., Krüger, D., Lueders, T., Scheu, S., Ruess, L. 2012, **Effects of resource availability and quality on the structure of the micro-food web of an arable soil across depth.** *Soil Biology & Biochemestry* 50:1.

2. **Dibbern D.**, Schmalwasser, A., Lueders T., Totsche, K. U. 2013 **Selective transport of plant root-associated bacterial populations in agricultural soils upon snowmelt.** *Soil Biology & Biochemestry* 69:187.

Pending manuscripts

3. Kramer S.*, **Dibbern D.***, Huenninghaus M., Moll J., Krueger D., Marhan S., Urich T., Wubet T., Bonkowski M., Buscot F., Lueders T., Kandeler E. **Eat all you can – Overlapping resource partitioning between bacteria, fungi and protists in the detritusphere.** *equal contribution (under final modification by co-authors, submitted to ISME Journal in October 2014)

4. **Dibbern D**., Hünninghaus, M., Koller, R., Engel, M., Bonkowski M., Lueders T. **Spatio-temporal distribution of key bacterial populations involved in a plant-derived soil food web in an agricultural field** (in preparation)

1. Scharroba *et al*.: Sampling of soil samples from July 2010 was done by all members of the Research Unit. Anika Scharroba (HU Berlin) investigated the nematodes, Maike Hünninghaus (U Köln) the protozoa, Julia Moll (UFZ Halle) the fungi from those samples. I analysed and generated the bacterial community data set and applied multivariate statistics (Multivariate regression tree) to the bacterial and fungal fingerprinting data sets. I also wrote respective sections of the manuscript text. These bacterial fingerprints from July 2010 are also part of the field monitoring presented in this thesis (Chapters 3.1 and 3.3).

2. Dibbern *et al.*: The experiment was planned by Dörte Dibbern and Tillmann Lueders. Kai Totsche and Andreas Schmalwasser (U Jena) established the lysimeter facilities and the latter also provided quantitative carbon data. REM pictures were developed in Kai

Totsches group. Soil samples were taken in the collaborative sampling campaign of the Reseach Unit, Lysimeter samples were taken at the same day by Dörte Dibbern and Andreas Schmalwasser. I generated and analysed the bacterial T-RFLP and amplicon pyrosequencing data sets and wrote most parts of the paper. Parts of the paper were used in the introduction, methods, results and discussion sections of this thesis regarding the transport of bacteria by seepage water (Chapters 1.3., 2.1.3, 3.4, 4.4).

3. Kramer *et al.*: The Experiment was planned by Susanne Kramer, Ellen Kandeler and Sven Marhan (U Hohenheim) together with Tillmann Lüders and Dörte Dibbern. Susanne Kramer conducted the experiment and wrote the first draft of the manuscript. I extracted soil RNA and performed rRNA-SIP analyses with help of Susanne Kramer. The fingerprinting and amplicon pyrosequencing data of the bacteria was performed and analysed by me, and I wrote respective parts of the manuscript. Micro-eukaryotic data sets (not shown in this thesis) were also generated by me but analysed by Maike Hünninghaus and Michael Bonkowski (U. Köln), who also wrote the respective parts of the manuscript. Julia Moll generated fungal data sets (not shown in this thesis) and analysed them together with Tesfaye Wubet (UFZ Halle), Dirk Krueger and Francois Buscot (UFZ Halle) wrote respective parts of the manuscript. Tillmann Lueders wrote, revised and edited large parts of the manuscript. Bacterial SIP data and some parts of the manuscript were used in the methods, results and discussion sections of this thesis regarding key detritusphere bacteria (Chapters 2.6.2, 3.2.2, 4.2.2).

4. Dibbern *et al.*: Soil samples were taken in the collaborative sampling campaign of the Reseach Unit. Field T-RFLP data sets and amplicon pyrosequencing (Chapters 3.1.2, 3.3.1) were generated and analysed by Dörte Dibbern, the latter with help of Marion Engel (Helmholtz München) and Tillmann Lueders. The rhizosphere SIP experiment was planned by Tillmann Lüders, Robert Koller and Michael Bonkowski together with Maike Hünninghaus and me. Maike Hünninghaus, Robert Koller, Dörte Dibbern and Julia Moll conducted the experiment. I generated and analysed T-RFLP data sets and amplicon pyrosequencing for the SIP experiment. The manuscript will be written largely by me and Tillmann Lüders, parts of this thesis regarding the distribution of identified bacterial food web members in the field (Chapters 3.3) and the rhizosphere SIP experiment (3.2.1) will be the basis for this manuscript.

Abbreviations

^{13}C	stable carbon isotope with standard atomic weight of 13
16S rRNA	ribosomal RNA, small subunit (bacteria)
bp	base pairs
DFG	German research funding organisation
DNA	deoxyribonucleic acid
DNA-SIP	stable isotope probing based on labelled DNA
dNTP	deoxyribonucleotide
e.g.	exempli gratia
et al.	et alii
exp.	experimental
fig.	figure
FOR 918	DFG Research unit 918 (‘Carbon flow in belowground food webs assessed by stable isotope tracers’)
HMGU	Helmholtz Zentrum München
i.e.	id est
MANOVA	multivariate analysis of variance
MRT	multivariate regression tree
*Msp*I	restriction enzyme of Moraxella sp. ATCC 49670
OTU	organizational taxonomic unit
PAH	polycyclic aromatic hydrocarbon
PCA	principal component analysis
PCR	polymerase chain reaction
PEG	polyethylene glycol
PGPR	Plant growth promoting rhizobacteria
qPCR	quantitative (=real time) polymerase chain reaction
RDA	redundancy analysis
RNA	ribonucleic acid
RNA-SIP	stable isotope probing based on labelled RNA
rRNA	ribosomal RNA
s. str.	sensu stricto
SIP	stable isotope probing
SOM	soil organic matter
spp.	species (plural)
*Taq*I	restriction enzyme of Thermus aquaticus
T-RF	terminal restriction fragment
T-RFLP	terminal restriction fragment length polymorphism
U	unit of enzyme activity, 1 µmol/min
Fo	functional organisation index
H'	Shannon-Weaver diversity index
PC1	first principal component
PC2	second principal component
TOC	total organic carbon
DOC	dissolved organic carbon
EOC	extractable organic carbon
TN	total nitrogen
POC	particulated organic carbon
PPI	sodium pyrophosphate
w/v	weight/volume
dw	dry weight
RT	reverse transcription
PGPR	plant growth promoting rhizobacteria
FAM	3′,6′-Dihydroxy-1-oxospiro[2-benzofuran- 3,9′-xanthen]-5-carbonsäure
ddH_2O	double-distilled water
whc	water holding capacity
i.s.	genera incerta sedis
uncl.	unclassified
s.g.	subgroup

Habt vielen Dank!

Meine Zeit als Doktorand habe ich als sehr reich empfunden: Freude und Frust lagen oft dicht beieinander, die zeitintensive Forschung musste mit Familie und Freizeit verbunden werden und ich wurde stark bereichert durch interessante Wissenschaft, neue Lebenserfahrungen und vielfältige Beziehungen. Es wird immer eine Zeit bleiben, an die ich viele Erinnerungen haben werde, wie auch an die Menschen, die mich auf dem Weg der Doktorarbeit begleitet und tatkräftig unterstützt haben.

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Lebenslauf

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